STIC Search Report
Biotech-Chem Library

STIC Database Tracking Number: 122661

To: Sarvamangala Devi **Location:** REM 3C18 **Art Unit:** 1645 Friday, May 21, 2004 **Case Serial Number:** 10/608873	**From:** Beverly Shears **Location:** Remsen Bldg. RM 1A54 **Phone:** 571-272-2528 beverly.shears@uspto.gov

Search Notes

122661

Shears, Beverly

From: Devi, Sarvamangala
Sent: Thursday, May 20, 2004 7:46 AM
To: Shears, Beverly
Subject: 10/608,873

Beverly:

Please perform a text search and in inventor's name search in application 10/608,873:

Claim 1. A method of decreasing the growth rate or reproduction rate of wild-type *Porphyromonas gingivalis* in a mnmmal, the method comprising administering to the mammal at least one dose of a non-virulent *recA*-defective mutant of Porphyromonas gingivalis.

Inventor: Hansel M. Fletcher

Thanx.

S. DEVI, Ph.D.
AU 1645
Rems - 3C18

STAFF USE ONLY

Date completed: 05-21-04
Searcher: Beverly C 2528
Terminal time: _____
Elapsed time: _____
CPU time: _____
Total time: _____
Number of Searches: _____
Number of Databases: 2

Search Site
_____ STIC
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Type of Search
_____ N.A. Sequence
_____ A.A. Sequence
_____ Structure
_____ Bibliographic

Vendors
_____ IG
✓ STN
✓ Dialog
_____ APS
_____ Geninfo
_____ SDC
_____ DARC/Questel
_____ Other

DeVi, S.
10/608873

FILE 'HCAPLUS' ENTERED AT 08:53:45 ON 21 MAY 2004
L1 2169 S (BACTERIOD? OR B OR PORPHYROM? OR P)(W)GINGIVAL? -Key Terms
L2 9 S L1 AND (RECA OR REC A)

L2 ANSWER 1 OF 9 HCAPLUS COPYRIGHT 2004 ACS on STN
ED Entered STN: 03 Oct 2003
ACCESSION NUMBER: 2003:775415 HCAPLUS
DOCUMENT NUMBER: 139:375984
TITLE: Multilocus sequence analysis of **Porphyromonas gingivalis** indicates frequent recombination
AUTHOR(S): Koehler, Andreas; Karch, Helge; Beikler, Thomas; Flemmig, Thomas F.; Suerbaum, Sebastian; Schmidt, Herbert
CORPORATE SOURCE: Institut fuer Hygiene und Mikrobiologie der Bayerischen Julius-Maximilians-Universitaet, Wuerzburg, 97080, Germany
SOURCE: Microbiology (Reading, United Kingdom) (2003), 149(9), 2407-2415
CODEN: MROBEO; ISSN: 1350-0872
PUBLISHER: Society for General Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB In this study, the genetic relationship of 19 **Porphyromonas gingivalis** isolates from patients with periodontitis was investigated by multilocus sequence anal. Internal 400-600 bp DNA fragments of the 10 chromosomal genes ef-tu, ftsQ, hagB, gpdxJ, pepO, mcmA, dnaK, **recA**, pga and nah were amplified by PCR and sequenced. No two isolates were identical at all 10 loci. Phylogenetic analyses indicated a panmictic population structure of **P. gingivalis**. Split decomposition anal., calcn. of homoplasy ratios and analyses of clustered polymorphisms all indicate that recombination plays a major role in creating the genetic heterogeneity of **P. gingivalis**. A standardized index of association of 0·0898 indicates that the **P. gingivalis** genes analyzed are close to linkage equilibrium
REFERENCE COUNT: 53 THERE ARE 53 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 2 OF 9 HCAPLUS COPYRIGHT 2004 ACS on STN
ED Entered STN: 14 Aug 2001
ACCESSION NUMBER: 2001:585983 HCAPLUS
DOCUMENT NUMBER: 136:197926
TITLE: The **recA** gene in **Porphyromonas gingivalis** is expressed during infection of the murine host
AUTHOR(S): Liu, Y.; Fletcher, H. M.
CORPORATE SOURCE: Department of Microbiology and Molecular Genetics, School of Medicine, Loma Linda University, Loma Linda, CA, 92350, USA
SOURCE: Oral Microbiology and Immunology (2001), 16(4), 218-223
CODEN: OMIMEE; ISSN: 0902-0055
PUBLISHER: Munksgaard International Publishers Ltd.

Devi, S.
10/608873

21may04 08:12:40 User219783 Session D2018.2

SYSTEM:OS - DIALOG OneSearch
File 65:Inside Conferences 1993-2004/May W3
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File 440:Current Contents Search(R) 1990-2004/May 21
(c) 2004 Inst for Sci Info
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File 113:European R&D Database 1997
(c)1997 Reed-Elsevier(UK)Ltd All rts reserv
*File 113: This file is closed (no updates)

Set	Items	Description

? ? ds; t 3/3,ab/1-8

-key terms

Set	Items	Description
S1	3633	(PORPHYROMON? OR P OR BACTERIOD? OR B)(W)GINGIVAL?
S2	40	S1 AND (RECA OR REC(W)A)
S3	8	RD (unique items)

>>>No matching display code(s) found in file(s): 65, 113

3/3,AB/1 (Item 1 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)
(c) 2004 Inst for Sci Info. All rts. reserv.

18386725 Document Delivery Available: 000221091100021 References: 48
TITLE: LuxS-mediated signaling in Streptococcus mutans is involved in regulation of acid and oxidative stress tolerance and biofilm formation
AUTHOR(S): Wen ZT; Burne RA (REPRINT)
AUTHOR(S) E-MAIL: rburne@dental.ufl.edu
CORPORATE SOURCE: Univ Florida, Coll Dent, Room D5-18/Gainesville//FL/32610 (REPRINT); Univ Florida, Coll Dent, /Gainesville//FL/32610
PUBLICATION TYPE: JOURNAL
PUBLICATION: JOURNAL OF BACTERIOLOGY, 2004, V186, N9 (MAY), P2682-2691
GENUINE ARTICLE#: 816DR
PUBLISHER: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA
ISSN: 0021-9193
LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: LuxS-mediated quorum sensing has recently been shown to regulate important physiologic functions and virulence in a variety of bacteria. In this study, the role of luxS of Streptococcus mutans in the regulation of traits crucial to pathogenesis was investigated. Reporter gene fusions showed that inactivation of luxS resulted in a down-regulation of fructanase, a demonstrated virulence determinant, by more than 50%. The LuxS-deficient strain (TW26) showed increased sensitivity to acid killing but could still undergo acid adaptation. Northern hybridization revealed that the expression of **RecA**, SmnA (AP endonuclease), and Nth (endonuclease) were down-regulated in TW26, especially in early-exponential-phase cells. Other down-regulated genes included ffh (a signal recognition particle subunit) and brpA (biofilm regulatory protein

A). Interestingly, the luxS mutant showed an increase in survival rate in the presence of hydrogen peroxide (58.8 mM). The luxS mutant formed less biofilm on hydroxylapatite disks, especially when grown in biofilm medium with sucrose, and the mutant biofilms appeared loose and hive-like, whereas the biofilms of the wild type were smooth and confluent. The mutant phenotypes were complemented by exposure to supernatants from wild-type cultures. Two loci, smu486 and smu487, were identified and predicted to encode a histidine kinase and a response regulator. The phenotypes of the smu486 smu487 mutant were, in almost all cases, similar to those of the luxS mutant, although our results suggest that this is not due to AI-2 signal transduction via Smu486 and Smu487. This study demonstrates that luxS-dependent signaling plays critical roles in modulating key virulence properties of S. mutans.

3/3,AB/2 (Item 2 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

17868129 Document Delivery Available: 000188892400005 References: 34
TITLE: The effect of oxygen on the growth and physiology of **Porphyromonas gingivalis**
AUTHOR(S): Diaz PI; Rogers AH (REPRINT)
AUTHOR(S) E-MAIL: tony.rogers@adelaide.edu.au
CORPORATE SOURCE: Univ Adelaide, Microbiol Lab, N Terrace/Adelaide/SA 5005/Australia/ (REPRINT); Univ Adelaide, Microbiol Lab, /Adelaide/SA 5005/Australia/
PUBLICATION TYPE: JOURNAL
PUBLICATION: ORAL MICROBIOLOGY AND IMMUNOLOGY, 2004, V19, N2 (APR), P88-94
GENUINE ARTICLE#: 773HK
PUBLISHER: BLACKWELL MUNKSGAARD, 35 NORRE SOGADE, PO BOX 2148, DK-1016 COPENHAGEN, DENMARK
ISSN: 0902-0055
LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: Oxygen constitutes a constant challenge for the survival of strict anaerobes in the oral environment. The aim of this study was to investigate the effect of oxygen on the physiology and growth of **Porphyromonas gingivalis** in a continuous culture system when grown under conditions of hemin limitation and excess. Results showed that, when grown in the presence of hemin at 0.5 mg/l, **P. gingivalis** could tolerate low levels of oxygen, being able to reach steady-state when 6% oxygen was present in the incoming gas mixture. When the hemin concentration was increased to 5 mg/l, the culture tolerated 10% oxygen. Anaerobically-grown cells were coccoid in shape, whereas those grown in the presence of oxygen were bacillary. Acetate was the predominant end-product in cultures grown in the presence of oxygen or in cultures hemin-limited. Despite some changes in the activity of Arg- and Lys-gingipain, most of the proteolytic activity was retained in the presence of oxygen. Activity of each of the three anti-oxidant enzymes tested (NADH oxidase, NADH peroxidase and SOD) was detected under all conditions and usually increased under oxygenated environments. Higher activities were also seen in the hemin-limited cultures. These results show some of the changes that occur in the physiology of **P. gingivalis** as a result of oxidative stress and confirm that hemin has a protective effect on the growth of the microorganism in the presence of oxygen.

3/3,AB/3 (Item 3 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

16932892 Document Delivery Available: 000185342900011 References: 53
TITLE: Multilocus sequence analysis of **Porphyromonas gingivalis** indicates frequent recombination
AUTHOR(S): Koehler A; Karch H; Beikler T; Flemmig TF; Suerbaum S; Schmidt H (REPRINT)
AUTHOR(S) E-MAIL: Herbert.Schmidt@mailbox.tu-dresden.de
CORPORATE SOURCE: Tech Univ Dresden, Inst Med Mikrobiol & Hyg, Fetscherstr 74/D-01307 Dresden//Germany/ (REPRINT); Univ Wurzburg, Inst Hyg & Mikrobiol, /D-97080 Wurzburg//Germany/; Univ Munster, Inst Hyg, /D-48149 Munster//Germany/; Univ Munster, Poliklin Parodontol, /D-48149 Munster//Germany/
PUBLICATION TYPE: JOURNAL
PUBLICATION: MICROBIOLOGY-SGM, 2003, V149, ,9 (SEP), P2407-2415
GENUINE ARTICLE#: 721XC
PUBLISHER: SOC GENERAL MICROBIOLOGY, MARLBOROUGH HOUSE, BASINGSTOKE RD, SPENCERS WOODS, READING RG7 1AG, BERKS, ENGLAND
ISSN: 1350-0872
LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: In this study, the genetic relationship of 19 **Porphyromonas gingivalis** isolates from patients with periodontitis was investigated by multilocus sequence analysis. Internal 400-600 bp DNA fragments of the 10 chromosomal genes ef-tu, ftsQ, hagB, gpdxJ, pepO, mcmA, dnaK, **recA** , pga and nah were amplified by PCR and sequenced. No two isolates were identical at all 10 loci. Phylogenetic analyses indicated a panmictic population structure of **P. gingivalis**. Split decomposition analysis, calculation of homoplasy ratios and analyses of clustered polymorphisms all indicate that recombination plays a major role in creating the genetic heterogeneity of **P. gingivalis**. A standardized index of association of 0.0898 indicates that the **P. gingivalis** genes analysed are close to linkage equilibrium.

3/3,AB/4 (Item 4 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

12897166 References: 28
TITLE: The **recA** gene in **Porphyromonas gingivalis** is expressed during infection of the murine host
AUTHOR(S): Liu Y; Fletcher HM (REPRINT)
CORPORATE SOURCE: Loma Linda Univ, Dept Microbiol & Mol Genet, /Loma Linda//CA/92350 (REPRINT); Loma Linda Univ, Dept Microbiol & Mol Genet, /Loma Linda//CA/92350
PUBLICATION TYPE: JOURNAL
PUBLICATION: ORAL MICROBIOLOGY AND IMMUNOLOGY, 2001, V16, N4 (AUG), P 218-223
GENUINE ARTICLE#: 454PF
PUBLISHER: MUNKSGAARD INT PUBL LTD, 35 NORRE SOGADE, PO BOX 2148, DK-1016 COPENHAGEN, DENMARK

ISSN: 0902-0055
LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: The **recA** gene in **Porphyromonas gingivalis** is involved in DNA repair. To further elucidate the importance of the **recA** locus in the pathogenesis of **P. gingivalis**, we assessed its ability for expression in an animal host. The promoterless xa-tet(Q)2 cassette was used in heterodiploid mutants to study **recA** promoter activity during infection. **P. gingivalis** FLL118.1 had the xa-tetA(Q)2 cassette under the control of **recA** promoter whereas **P. gingivalis** FLL119 had the cassette in the opposite orientation. xa encodes a bifunctional xylosidase/arabinosidase enzyme (XA) and the tetA(Q)2 gene product confers tetracycline resistance. Intramuscular infection in a mouse model allowed the recovery of the bacteria from inguinal lymph nodes. Infusion of tetracycline in the animals permitted the enrichment **P. gingivalis** FLL118.1 over the wild-type strain, during a mixed infection. The xylosidase activity of FLL118.1 could be detected on agar plates in the presence of 5-methylumbellifiry-beta -D-xyloside. No such enrichment for xylosidase activity was detected when the mixture of **P. gingivalis** W83 and **P. gingivalis** FLL119 was used to infect the mouse or cultured in vitro. These results indicated that **recA** promoter was transcriptionally active during the infection of the murine host and further support the importance of this locus during the **P. gingivalis** infection process.

3/3,AB/5 (Item 5 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

12684680 References: 40
TITLE: Environmental regulation of **recA** gene expression in **Porphyromonas gingivalis**
AUTHOR(S): Liu Y; Fletcher HM (REPRINT)
CORPORATE SOURCE: Loma Linda Univ, Dept Microbiol & Mol Genet, /Loma Linda//CA/92350 (REPRINT); Loma Linda Univ, Dept Microbiol & Mol Genet, /Loma Linda//CA/92350
PUBLICATION TYPE: JOURNAL
PUBLICATION: ORAL MICROBIOLOGY AND IMMUNOLOGY, 2001, V16, N3 (JUN), P 136-143
GENUINE ARTICLE#: 430HD
PUBLISHER: MUNKSGAARD INT PUBL LTD, 35 NORRE SOGADE, PO BOX 2148, DK-1016 COPENHAGEN, DENMARK
ISSN: 0902-0055
LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: The **recA** gene product in **Porphyromonas gingivalis** is involved in DNA repair. Further, disruption of this gene can affect the proteolytic activity and expression of other virulence factors in this organism. Since several known environmental factors can influence virulence gene expression in **P. gingivalis**, we investigated the influence of these signals on the expression of the **recA** gene in this organism. A heterodiploid strain of **P. gingivalis** (designated FLL 118) containing a transcriptional fusion of the **recA** promoter region and the promoterless

tetracycline-resistant gene [tetA (Q)2] and xylosidase/arabinosidase (xa) gene cassette was constructed. The **recA** promoter activity was assessed by measurement of xylosidase activity in FLL118. The expression remained relatively constant during different growth phases, at different pH levels and in the presence of DNA-damaging agents. In response to hemin limitation and in the presence of calcium there was a moderate increase in **recA** promoter activity. Temperature also affected the expression. The highest level of xylosidase activity was observed in cultures at 32 degreesC with a decline of approximately 46% as growth temperature increased to 41 degreesC. Reverse transcriptase polymerase chain reaction analysis revealed that this regulation may be occurring at the transcriptional level. These results suggest that expression of the **recA** gene in **P. gingivalis** W83 is responsive to several environmental signals but is not regulated by a DNA damage-inducible SOS-like regulatory system.

3/3,AB/6 (Item 6 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

12271544 References: 66
TITLE: vimA gene downstream of **recA** is involved in virulence modulation in **Porphyromonas gingivalis** W83
AUTHOR(S): Abaibou H; Chen Z; Olango GJ; Liu Y; Edwards J; Fletcher HM (REPRINT)
AUTHOR(S) E-MAIL: HFLETCHER@SOM.LLU.EDU
CORPORATE SOURCE: Loma Linda Univ, Dept Microbiol & Mol Genet, /Loma Linda//CA/92350 (REPRINT); Loma Linda Univ, Dept Microbiol & Mol Genet, /Loma Linda//CA/92350
PUBLICATION TYPE: JOURNAL
PUBLICATION: INFECTION AND IMMUNITY, 2001, V69, N1 (JAN), P325-335
GENUINE ARTICLE#: 384LF
PUBLISHER: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA
ISSN: 0019-9567
LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: A 0.9-kb open reading frame encoding a unique 32-kDa protein was identified downstream of the **recA** gene of **Porphyromonas gingivalis**. Reverse transcription PCR and Northern blot analysis showed that both the **recA** gene and this open reading frame are part of the same transcriptional unit. This cloned fragment was insertionally inactivated using the ermF-ermAM antibiotic resistance cassette to create a defective mutant by allelic exchange. When plated on Brucella blood agar, the mutant strain, designated **P. gingivalis** FLL92, was non-black pigmented and showed significant reduction in beta-hemolysis compared with the parent strain, **P. gingivalis** W83. Arginine- and lysine-specific cysteine protease activities, which were mostly soluble, were approximately 90% lower than that of the parent strain. Expression of the rgpA, rgpB, and kgp protease genes was the same in **P. gingivalis** FLL92 as in the wild-type strain. In contrast to the parent strain, **P. gingivalis** FLL92 showed increased autoaggregration in addition to a significant reduction in hemagglutinating and hemolysin activities. In in vivo experiments using a mouse model, **P. gingivalis** FLL92 was dramatically less virulent than the

parent strain. A molecular survey of this mutant and the parent strain using all known **P. gingivalis** insertion sequence elements as probes suggested that no intragenomic changes due to the movement of these elements have occurred in **P. gingivalis** FLL92. Taken together, these results suggest that the **recA** downstream gene, designated vimA (virulence-modulating gene), plays an important role in virulence modulation in **P. gingivalis** W83, possibly representing a novel posttranscriptional or translational regulation of virulence factors in **P. gingivalis**.

3/3,AB/7 (Item 7 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

11285045 References: 34
TITLE: Unaltered expression of the major protease genes in a non-virulent **recA**-defective mutant of **Porphyromonas gingivalis** W83
AUTHOR(S): Abaibou H; Ma Q; Olango GJ; Potempa J; Travis J; Fletcher HM (REPRINT)
CORPORATE SOURCE: Loma Linda Univ, Dept Microbiol & Mol Genet, /Loma Linda//CA/92350 (REPRINT); Loma Linda Univ, Dept Microbiol & Mol Genet, /Loma Linda//CA/92350; Jagiellonian Univ, Dept Microbiol & Immunol, /Krakow//Poland/; Univ Georgia, Dept Biochem, /Athens//GA/30602
PUBLICATION TYPE: JOURNAL
PUBLICATION: ORAL MICROBIOLOGY AND IMMUNOLOGY, 2000, V15, N1 (FEB), P40-47
GENUINE ARTICLE#: 277EC
PUBLISHER: MUNKSGAARD INT PUBL LTD, 35 NORRE SOGADE, PO BOX 2148, DK-1016 COPENHAGEN, DENMARK
ISSN: 0902-0055
LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: **Porphyromonas gingivalis** FLL32, a **recA** mutant, was isolated during construction of a **recA** defective mutant of **P. gingivalis** W83 by allelic exchange mutagenesis. In contrast to W83 and FLL33, the typical **recA**(-) mutant previously reported, FLL32 was non-pigmented, lacked beta-hemolytic activity an blood agar and produced significantly less proteolytic activity. The proteolytic activity in FLL32 was mostly soluble. Expression of the rgpA, rgpB and kgp protease genes was unaltered in FLL32 when compared to FLL33 and the wild-type strain. FLL32 exhibited reduced virulence in a murine model and partially protected the animals immunized with that strain against a subsequent lethal challenge by the wild-type strain. These results indicate that the reduced level of proteolytic activity in FLL32 may be due to a defect in the processing of the proteases. Further, immunization with a non-virulent **recA** defective mutant of **P. gingivalis** can partially protect against a lethal wild-type challenge. The results from this study suggest that the **recA** locus may be involved in expression and regulation of proteolytic activity in **P. gingivalis**.

3/3,AB/8 (Item 8 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

08936067 References: 39

TITLE: Nucleotide sequence of the **Porphyromonas gingivalis** W83 **recA** homolog and construction of a **recA**-deficient mutant
AUTHOR(S): Fletcher HM (REPRINT); Morgan RM; Macrina FL
CORPORATE SOURCE: LOMA LINDA UNIV,DEPT MOL GENET & MICROBIOL/LOMA LINDA//CA/92350 (REPRINT); VIRGINIA COMMONWEALTH UNIV,DEPT MICROBIOL & IMMUNOL/RICHMOND//VA/23298
PUBLICATION TYPE: JOURNAL
PUBLICATION: INFECTION AND IMMUNITY, 1997, V65, N11 (NOV), P4592-4597
GENUINE ARTICLE#: YD176
PUBLISHER: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171
ISSN: 0019-9567
LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: Degenerate oligonucleotide primers were used in PCR to amplify a region of the **recA** homolog-from **Porphyromonas gingivalis** W83., The resulting PCR fragment was used as a probe to identify a recombinant lambda DASH phage (L10) carrying the **P. gingivalis recA** homolog. The **recA** homolog was localized to a 2.1-kb BamHI fragment. The nucleotide sequence of this 2.1-kb fragment was determined, and a 1.02-kb open reading frame (341 amino acids) was detected. The predicted amino acid sequence was strikingly similar (90% identical residues) to the **RecA** protein from Bacteroides fragilis, No SOS box, characteristic of LexA-regulated promoters, was found in the 5' upstream region of the **P. gingivalis recA** homolog, In both methyl methanesulfonate and UV survival experiments the **recA** homolog from **P. gingivalis** complemented the **recA** mutation of Escherichia coli HB101. The cloned **P. gingivalis recA** gene was insertionally inactivated with the ermF-ermAM antibiotic resistance cassette to create a **recA**-deficient mutant (FLL33) by allelic exchange. The **recA**-deficient mutant was significantly more sensitive to UV irradiation than the wild-type strain, W83. W83 and FLL33 showed the same level of virulence in in vivo experiments using a mouse model. These results suggest that the **recA** gene in **P. gingivalis** W83 plays the expected role of repairing DNA damage caused by UV irradiation. However, inactivation of this gene did not alter the virulence of **P. gingivalis** in the mouse model.

Set	Items	Description
S4	239	AU=(FLETCHER, H? OR FLETCHER H?)
S5	25	S1 AND S4
S6	15	S5 NOT S2
S7	8	RD (unique items)

– Author

>>>No matching display code(s) found in file(s): 65, 113

7/3,AB/1 (Item 1 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

16447304 Document Delivery Available: 000183797200009 References: 44
TITLE: Gingipain RgpB is excreted as a proenzyme in the vimA-defective mutant **Porphyromonas gingivalis** FLL92
AUTHOR(S): Olango GJ; Roy F; Sheets SM; Young MK; **Fletcher HM (REPRINT)**
AUTHOR(S) E-MAIL: hfletcher@som.llu.edu
CORPORATE SOURCE: Loma Linda Univ, Div Microbiol & Mol Genet, /Loma

Linda//CA/92350 (REPRINT); Loma Linda Univ, Div Microbiol & Mol Genet, /Loma Linda//CA/92350; City Hope Natl Med Ctr, Div Immunol, /Duarte//CA/91010
PUBLICATION TYPE: JOURNAL
PUBLICATION: INFECTION AND IMMUNITY, 2003, V71, N7 (JUL), P3740-3747
GENUINE ARTICLE#: 694WB
PUBLISHER: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA
ISSN: 0019-9567
LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: We have previously shown that the unique vimA (virulence-modulating) gene could modulate proteolytic activity in Porphyromomas gingivalis. Although a reduction in cysteine protease activity was observed in the vimA-defective mutant, **P. gingivalis** FLL92, compared to that of the wild-type strain, no changes were seen in the expression of the gingipain genes. This result might suggest posttranscriptional regulation of protease expression. To determine whether there was a defect in the translation, transport, or maturation of the gingipains, **P. gingivalis** FLL92 was further characterized. In contrast to the wild-type strain, a 90% reduction was seen in both Rgp and Kgp protease activities in strain FLL92 during the exponential growth phase. These activities, however, increased, to approximately 60% of that of the wild-type strain during stationary phase. Throughout all the growth phases, Rgp and Kgp activities were mostly soluble, in contrast to those of the wild-type strain. Western blot analyses identified unique Rgp- and Kgp-immunoreactive bands in extracellular protein fractions from FLL92 grown to late exponential phase. Also, the RgpB proenzyme was identified in this fraction by mass spectrometry. In addition, in vitro protease activity could be induced by a urea denaturation-renaturation cycle in this fraction. These results indicate that protease activity in **P. gingivalis** may be growth phase regulated, possibly by multiple mechanisms. Furthermore, the gingipain RgpB is excreted in an inactive form in the vimA mutant. In addition, these results provide the first evidence of posttranslational regulation of protease activity in **P. gingivalis** and may suggest an important role for the vimA gene in protease activation in this organism.

7/3,AB/2 (Item 2 from file: 440)

14050297 Document Delivery Available: 000176033500023 References: 56
TITLE: Accelerated alveolar bone loss in HLA-B27 transgenic rats: An adult onset condition
AUTHOR(S): Tatakis DN (REPRINT); Guglielmoni P; **Fletcher HM**
AUTHOR(S) E-MAIL: tatakis.1@osu.edu
CORPORATE SOURCE: Ohio State Univ, Sect Periodontol, 305 W 12th Ave,POB 182357/Columbus//OH/43218 (REPRINT); Loma Linda Univ, Dept Periodont, /Loma Linda//CA/92350; Loma Linda Univ, Dept Microbiol & Mol Genet, /Loma Linda//CA/92350
PUBLICATION TYPE: JOURNAL
PUBLICATION: JOURNAL OF RHEUMATOLOGY, 2002, V29, N6 (JUN), P1244-1251
GENUINE ARTICLE#: 559PA

PUBLISHER: J RHEUMATOL PUBL CO, 920 YONGE ST, SUITE 115, TORONTO, ONTARIO M4W 3C7, CANADA
ISSN: 0315-162X
LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: Objective. Patients with arthritis and Crohn's disease may be more susceptible to periodontitis associated alveolar bone loss (ABL). HLA-B27 transgenic (TG) rats spontaneously develop arthritis and colitis. Based on the hypothesis that TG rats would also be susceptible to ABL, we compared the naturally occurring ABL in TG and Fischer 344 wild-type (WT) rats.

Methods. Eighteen TG and 18 WT virgin female rats were used. Pairs (1 TG, 1 WT) were housed in suspended wire cages. At age 2.6, 6, and 11 months, 8, 5, and 5 pairs were sacrificed, respectively. ABL was measured as exposed molar root surface area (mm(2)). Western blotting was used for analysis of serum reactivity against bacteria associated with arthritis, colitis, and periodontitis development.

Results. At 2.6 months of age, there was no difference in ABL between TG and WT rats. At 6 and I I months ABL was significantly greater in TG animals by 28% and 53%, respectively. For TG rats, ABL was significantly different between the 3 age groups. For WT rats, ABL was not significantly different between 6 and 11 months. Western blotting revealed distinct TG serum reactivity against extracts of Bacteroides vulgatus, B. fragilis, Prevotella intermedia, and to a lesser extent against extracts of B. forsythus.

Conclusion. The accelerated ABL in HLA-B27 TG rats is an adult onset condition, independent of husbandry conditions or parity status. HLA-B27 rats exhibit strong immunoreactivity against bacteria implicated in arthritis, colitis, and periodontitis.

7/3,AB/3 (Item 3 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

12745090 References: 48
TITLE: Protease-active extracellular protein preparations from **Porphyromonas gingivalis** W83 induce N-cadherin proteolysis, loss of cell adhesion, and apoptosis in human epithelial cells
AUTHOR(S): Chen Z; Casiano CA; **Fletcher HM (REPRINT)**
AUTHOR(S) E-MAIL: hfletcher@som.llu.edu
CORPORATE SOURCE: Loma Linda Univ, Dept Microbiol & Mol Genet, /Loma Linda//CA/92350 (REPRINT); Loma Linda Univ, Dept Microbiol & Mol Genet, /Loma Linda//CA/92350; Loma Linda Univ, Ctr Mol Biol & Gene Therapy, /Loma Linda//CA/92350
PUBLICATION TYPE: JOURNAL
PUBLICATION: JOURNAL OF PERIODONTOLOGY, 2001, V72, N5 (MAY), P641-650
GENUINE ARTICLE#: 435XZ
PUBLISHER: AMER ACAD PERIODONTOLOGY, 737 NORTH MICHIGAN AVENUE, SUITE 800, CHICAGO, IL 60611-2690 USA
ISSN: 0022-3492
LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: Background: The protease-induced cytotoxicity of **P. gingivalis** may partly result from alteration of the extracellular matrix and/or surface receptors that mediate interaction between the host cells and their matrix. While **P. gingivalis**-induced degradation of E-cadherin has been documented, there is no information on the effects of **P. gingivalis** proteases on other members of this family of cell adhesion proteins.

Methods: Human epithelial KB cells were exposed to protease-active extracellular protein preparations from isogenic mutants of **P. gingivalis**. Quantification of apoptosis was performed by visualization of nuclei stained with 4,6 ' -diamidino-2-phenylindole. Alteration of cell adhesion proteins was examined by immunoblotting of cell lysates using monoclonal antibodies to those proteins.

Results: Treated cells exhibited loss of cell adhesion properties with apoptotic cell death subsequently observed. These effects correlated with the different levels of cysteine-dependent proteolytic activities of the isogenic mutants tested. Cleavage of N-cadherin was observed in immunoblots of lysates from detached cells. There was a direct correlation between the kinetics of N-cadherin cleavage and loss of cell adhesion properties. Loss of cell adhesion, as well as N-cadherin cleavage, could be inhibited by preincubation of **P. gingivalis** protease active extracellular protein preparations with the cysteine protease inhibitor TLCK. In control experiments, the cleavage of N-cadherin was detected after treatment of KB cells with trypsin but not after cell dissociation by a non-enzymatic method.

Conclusions: These results suggest that extracellular pro teases from **P. gingivalis** can induce degradation of N-cadherin, which could have implications for the pathogenicity of this bacterium.

7/3,AB/4 (Item 4 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

12217240 References: 28
TITLE: Development of a noninvasive reporter system for gene expression in **Porphyromonas gingivalis**
AUTHOR(S): Liu Y; Abaibou H; **Fletcher HM (REPRINT)**
AUTHOR(S) E-MAIL: HFLETCHER@SOM.LLU.EDU
CORPORATE SOURCE: Loma Linda Univ, Dept Microbiol & Mol Genet, /Loma Linda//CA/92350 (REPRINT); Loma Linda Univ, Dept Microbiol & Mol Genet, /Loma Linda//CA/92350
PUBLICATION TYPE: JOURNAL
PUBLICATION: PLASMID, 2000, V44, N3 (NOV), P250-261
GENUINE ARTICLE#: 378WK
PUBLISHER: ACADEMIC PRESS INC, 525 B ST, STE 1900, SAN DIEGO, CA 92101-4495 USA
ISSN: 0147-619X
LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: Several reports have supported the association of **Porphyromonas gingivalis** with periodontal disease. Genetic studies are vital for understanding the relative importance of virulence

factors in this organism. Thus, gene reporters may prove useful for the study of gene expression in this organism. We have investigated the use of the green fluorescent protein (GTP), bacterial luciferase, and bifunctional xylosidase/arabinosidase enzyme (XA) as reporters of gene expression in **P. gingivalis**. Fusion cassettes containing the promoterless tetracycline resistant gene [tetA(A)Q2] and the promoterless gfp, luxAB, or xa gene were placed under the control of the rgpA promoter in **P. gingivalis** W83 using recombinational allelic exchange. The rgpA gene encodes for an arginine-specific pretense in **P. gingivalis**. No GFP activity was detected in **P. gingivalis** isogenic mutants carrying the rgpA::gfp-tetA(Q)2 fusion construct. Luciferase activity in **P. gingivalis** mutants carrying the rgpA::luxAB-tetA(Q)2 fusion was only detected in the presence of exogenous FMNH2. xa gene expression in **P. gingivalis** with the rgpA::xa-tetA(Q)2 fusion construct was detected in crude extracts using rho -nitrophenol derivatives as substrate and on agar plates with methylumbelliferyl derivatives under long-wave ultraviolet light. This indicates that both luxAB and xa genes can be used as reporters of gene expression in **P, gingivalis**. However, only the xa gene can be used as a noninvasive reporter gene. (C) 2000 Academic Press.

7/3,AB/5 (Item 5 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

06365022 References: 33
TITLE: INCREASED OPSONIZATION OF A PRTH-DEFECTIVE MUTANT OF **PORPHYROMONAS GINGIVALIS** W83 IS CAUSED BY REDUCED DEGRADATION OF COMPLEMENT-DERIVED OPSONINS
AUTHOR(S): SCHENKEIN HA; **FLETCHER HM**; BODNAR M; MACRINA FL
CORPORATE SOURCE: VIRGINIA COMMONWEALTH UNIV,SCH DENT,CLIN PERIODONTAL DIS RES CTR,MCV STN BOX 980566/RICHMOND//VA/23298 (Reprint); VIRGINIA COMMONWEALTH UNIV,SCH MED,DEPT MICROBIOL & IMMUNOL/RICHMOND//VA/23298
PUBLICATION: JOURNAL OF IMMUNOLOGY, 1995, V154, N10 (MAY 15), P5331-5337
GENUINE ARTICLE#: QW901
ISSN: 0022-1767
LANGUAGE: ENGLISH DOCUMENT TYPE: ARTICLE

ABSTRACT: Periodontitis is a disease of the supporting structures of the teeth that is caused by bacteria whose common ecologic niche is the gingival crevicé or the periodontal pocket. Tissue destruction occurs in spite of both local and systemic immune responses against such bacteria. **Porphyromonas gingivalis** is considered to be an important pathogen in some forms of human periodontitis and is particularly interesting because of its multiplicity of virulence factors. We have previously observed that phagocytosis-resistant invasive strains of **P. gingivalis** proteolytically degrade C3 and IgG and accumulate less C3-derived opsonins during complement activation. We recently have cloned the prtH gene from **P. gingivalis** W83 that encodes a 97-kDa active protease, which has the capacity to degrade purified C3 protein. By using this cloned gene we created an allelic exchange mutant of **P. gingivalis** W83, designated V2296, in which the prtH gene was inactivated. This mutant was previously shown to be less virulent than its parent strain W83 in a mouse model of bacterial invasiveness. In the present study we have assessed the relative capacity of V2296 and W83 to be

opsonized by complement and to be taken up by PMNs. The data demonstrate that V2296, in comparison with its parent strain W83, is less able to degrade C3 and that it accumulates significantly greater numbers of molecules of C3-derived opsonins on the bacterial surface in the form of C3b and iC3b during complement activation. Furthermore, opsonized V2296 is taken up in much higher numbers by human PMNs than W83, suggesting that the prtH gene product may be important in evasion of host defense mechanisms.

7/3,AB/6 (Item 6 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

06272765 References: 48
TITLE: VIRULENCE OF A **PORPHYROMONAS GINGIVALIS** W83 MUTANT DEFECTIVE IN THE PRTH GENE
AUTHOR(S): **FLETCHER HM**; SCHENKEIN HA; MORGAN RM; BAILEY KA; BERRY CR; MACRINA FL
CORPORATE SOURCE: VIRGINIA COMMONWEALTH UNIV,DEPT MICROBIOL & IMMUNOL/RICHMOND//VA/23298 (Reprint); VIRGINIA COMMONWEALTH UNIV,CLIN RES CTR PERIODONTAL DIS/RICHMOND//VA/23298
PUBLICATION: INFECTION AND IMMUNITY, 1995, V63, N4 (APR), P1521-1528
GENUINE ARTICLE#: QP134
ISSN: 0019-9567
LANGUAGE: ENGLISH DOCUMENT TYPE: ARTICLE

ABSTRACT: In a previous study we cloned and determined the nucleotide sequence of the prtH gene from **Porphyromonas gingivalis** W83. This gene specifies a 97-kDa protease which is normally found in the membrane vesicles produced by **P. gingivalis** and which cleaves the C3 complement protein under defined conditions. We developed a novel ermF-ermAM antibiotic resistance gene cassette, which was used with the cloned prtH gene to prepare an insertionally inactivated allele of this gene. This genetic construct was introduced by electroporation into **P. gingivalis** W83 in order to create a protease-deficient mutant by recombinational allelic exchange. The mutant strain, designated V2296, was compared with the parent strain W83 for proteolytic activity and virulence. Extracellular protein preparations from V2296 showed decreased proteolytic activity compared with preparations from W83. Casein substrate zymography revealed that the 97-kDa proteolytic component as well as a 45-kDa protease was missing in the mutant, In in vivo experiments using a mouse model, V2296 was dramatically reduced in virulence compared with the wild-type W83 strain. A molecular survey of several clinical isolates of **P. gingivalis** using the prtH gene as a probe suggested that prtH gene sequences were conserved and that they may have been present in multiple copies. Two of 10 isolates did not hybridize with the prtH gene probe, These strains, like the V2296 mutant, also displayed decreased virulence in the mouse model. Taken together, these results suggest an important role for **P. gingivalis** proteases in soft tissue infections and specifically indicate that the prtH gene product is a virulence factor.

7/3,AB/7 (Item 7 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

05943912 References: 1
TITLE: CLONING AND CHARACTERIZATION OF A NEW PROTEASE GENE (PRTH) FROM **PORPHYROMONAS GINGIVALIS** (VOL 62, PG 4281, 1994)
AUTHOR(S): **FLETCHER HM**; SCHENKEIN HA; MACRINA FL
CORPORATE SOURCE: VIRGINIA COMMONWEALTH UNIV,DEPT MICROBIOL & IMMUNOL/RICHMOND//VA/23298 (Reprint); VIRGINIA COMMONWEALTH UNIV,CLIN RES CTR PERIODONTAL DIS/RICHMOND//VA/23298
PUBLICATION: INFECTION AND IMMUNITY, 1994, V62, N12 (DEC), P5707
GENUINE ARTICLE#: PT329
ISSN: 0019-9567
LANGUAGE: ENGLISH DOCUMENT TYPE: CORRECTION, ADDITION

7/3,AB/8 (Item 8 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

05784444 References: 41
TITLE: CLONING AND CHARACTERIZATION OF A NEW PROTEASE GENE (PRTH) FROM **PORPHYROMONAS GINGIVALIS**
AUTHOR(S): **FLETCHER HM**; SCHENKEIN HA; MACRINA FL
CORPORATE SOURCE: VIRGINIA COMMONWEALTH UNIV,DEPT MICROBIOL & IMMUNOL/RICHMOND//VA/23298 (Reprint); VIRGINIA COMMONWEALTH UNIV,CLIN RES CTR PERIODONTAL DIS/RICHMOND//VA/23298
PUBLICATION: INFECTION AND IMMUNITY, 1994, V62, N10 (OCT), P4279-4286
GENUINE ARTICLE#: PH298
ISSN: 0019-9567
LANGUAGE: ENGLISH DOCUMENT TYPE: ARTICLE

ABSTRACT: **Porphyromonas gingivalis** has been implicated as a contributing etiological agent of adult periodontitis and generalized forms of early-onset periodontitis. Proteases of **P. gingivalis** may contribute to its pathogenicity by destroying connective tissue as well as inactivating keg plasma proteins that might mediate protective host functions. In order to explore this problem, antiserum raised against membrane vesicles of **P. gingivalis** W83 was used to screen a genomic library of strain W83 constructed by using the lambda DASH vector system. A recombinant phage (lambda 34) expressing a **P. gingivalis** protease from the library was identified and characterized. Casein substrate zymography of lambda 34 lysates revealed a protease with an apparent molecular mass of 97 kDa. The gene encoding this protease was designated prtH. It was localized to a 3.7-kb HindIII-BamHI fragment and specified an enzyme which hydrolyzed the human C3 complement protein under defined conditions. The nucleotide sequence of this 3.7-kb fragment was determined, and one 2.9-kb open reading frame (992 amino acids) corresponding to a 110-kDa protein was detected, suggesting it might be a precursor of the 97-kDa active protease. prtH is not similar to any previously cloned protease gene from **P. gingivalis**.
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21may04 08:15:16 User219783 Session D2018.3

DOCUMENT TYPE: Journal
LANGUAGE: English
AB The **recA** gene in **P. gingivalis** is involved in DNA repair. To further elucidate the importance of the **recA** locus in the pathogenesis of **P. gingivalis**, the authors assessed its ability for expression in an animal host. The promoterless xa-tetA(Q)2 cassette was used in heterodiploid mutants to study **recA** promoter activity during infection. **P. gingivalis** FLL118.1 had the xa-tetA(Q)2 cassette under the control of **recA** promoter whereas **P. gingivalis** FLL119 had the cassette in the opposite orientation. Xa encodes a bifunctional xylosidase/arabinosidase enzyme (XA) and the tetA(Q)2 gene product confers tetracycline resistance. I.m. infection in a mouse model allowed the recovery of the bacteria from inguinal lymph nodes. Infusion of tetracycline in the animals permitted the enrichment **P. gingivalis** FLL118.1 over the wild-type strain, during a mixed infection. The xylosidase activity of FLL118.1 could be detected on agar plates in the presence of 5-methylumbellifiry-β-D-xyloside. No such enrichment for xylosidase activity was detected when the mixture of **P. gingivalis** W83 and **P. gingivalis** FLL119 was used to infect the mouse or cultured in vitro. These results indicated that **recA** promoter was transcriptionally active during the infection of the murine host and further support the importance of this locus during the **P. gingivalis** infection process.
REFERENCE COUNT: 28 THERE ARE 28 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 3 OF 9 HCAPLUS COPYRIGHT 2004 ACS on STN
ED Entered STN: 05 Jun 2001
ACCESSION NUMBER: 2001:399932 HCAPLUS
DOCUMENT NUMBER: 136:145959
TITLE: Environmental regulation of **recA** gene expression in **Porphyromonas gingivalis**
AUTHOR(S): Liu, Y.; Fletcher, H. M.
CORPORATE SOURCE: Department of Microbiology and Molecular Genetics, School of Medicine, Loma Linda University, Loma Linda, CA, 92350, USA
SOURCE: Oral Microbiology and Immunology (2001), 16(3), 136-143
CODEN: OMIMEE; ISSN: 0902-0055
PUBLISHER: Munksgaard International Publishers Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The **recA** gene product in **Porphyromonas gingivalis** is involved in DNA repair. Further, disruption of this gene can affect the proteolytic activity and expression of other virulence factors in this organism. Since several known environmental factors can influence virulence gene expression in **P. gingivalis**, we investigated the influence of these signals on the expression of the **recA** gene in this organism. A heterodiploid strain of **P. gingivalis** (designated FLL118) containing a transcriptional fusion of the

recA promoter region and the promoterless tetracycline-resistant gene [tetA (Q)2] and xylosidase/arabinosidase (xa) gene cassette was constructed. The **recA** promoter activity was assessed by measurement of xylosidase activity in FLL118. The expression remained relatively constant during different growth phases, at different pH levels and in the presence of DNA-damaging agents. In response to hemin limitation and in the presence of calcium there was a moderate increase in **recA** promoter activity. Temperature also affected the expression. The highest level of xylosidase activity was observed in cultures at 32°C with a decline of approx. 46% as growth temperature increased to 41°C. Reverse transcriptase polymerase chain reaction anal. revealed that this regulation may be occurring at the transcriptional level. These results suggest that expression of the **recA** gene in **P. gingivalis** W83 is responsive to several environmental signals but is not regulated by a DNA damage-inducible SOS-like regulatory system.

REFERENCE COUNT: 41 THERE ARE 41 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 4 OF 9 HCAPLUS COPYRIGHT 2004 ACS on STN
ED Entered STN: 06 Apr 2001
ACCESSION NUMBER: 2001:247534 HCAPLUS
DOCUMENT NUMBER: 134:291065
TITLE: Highly conserved genes and their use to generate species-specific, genus-specific, family-specific, group-specific and universal nucleic acid probes and amplification primers to rapidly detect and identify algal, archaeal, bacterial, fungal and parasitical microorganismsfrom clinical specimens for diagnosis
INVENTOR(S): Bergeron, Michel G.; Boissinot, Maurice; Huletsky, Ann; Menard, Christian; Ouellette, Marc; Picard, Francois J.; Roy, Paul H.
PATENT ASSIGNEE(S): Infectio Diagnostic (I.D.I.) Inc., Can.
SOURCE: PCT Int. Appl., 1580 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001023604	A2	20010405	WO 2000-CA1150	20000928
WO 2001023604	A3	20020808		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH,

CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE,
BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG
EP 1246935 A2 20021009 EP 2000-965686 20000928
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC,
PT, IE, SI, LT, LV, FI, RO, MK, CY, AL
BR 2000014370 A 20021105 BR 2000-14370 20000928
JP 2003511015 T2 20030325 JP 2001-526986 20000928
PRIORITY APPLN. INFO.: CA 1999-2283458 A 19990928
CA 2000-2307010 A 20000519
WO 2000-CA1150 W 20000928

AB Four highly conserved genes encoding translation elongation factor Tu, translation elongation factor G, the catalytic subunit of proton-translocating ATPase, and **RecA** recombinase are used to generate a sequence repertory or bank and species-specific, genus-specific, family-specific, group-specific and universal nucleic acid probes and amplification primers to rapidly detect and identify algal, archaeal, bacterial, fungal, and parasitical microorganisms from specimens for diagnosis. The detection of associated antimicrobial agent resistance and toxin genes is also under the scope of the present invention.

L2 ANSWER 5 OF 9 HCAPLUS COPYRIGHT 2004 ACS on STN
ED Entered STN: 10 Jan 2001
ACCESSION NUMBER: 2001:20261 HCAPLUS
DOCUMENT NUMBER: 134:190463
TITLE: vimA gene downstream of **recA** is involved in virulence modulation in **Porphyromonas gingivalis** W83
AUTHOR(S): Abaibou, Hafid; Chen, Zhuo; Olango, G. Jon; Liu, Yi; Edwards, Jessica; Fletcher, Hansel M.
CORPORATE SOURCE: Department of Microbiology and Molecular Genetics, School of Medicine, Loma Linda University, Loma Linda, CA, 92350, USA
SOURCE: Infection and Immunity (2001), 69(1), 325-335
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A 0.9-kb open reading frame encoding a unique 32-kDa protein was identified downstream of the **recA** gene of **Porphyromonas gingivalis**. Reverse transcription-PCR and Northern blot anal. showed that both the **recA** gene and this open reading frame are part of the same transcriptional unit. This cloned fragment was insertionally inactivated using the ermF-ermAM antibiotic resistance cassette to create a defective mutant by allelic exchange. When plated on Brucella blood agar, the mutant strain, designated **P. gingivalis** FLL92, was non-black pigmented and showed significant reduction in beta-hemolysis compared with the parent strain, **P. gingivalis** W83. Arginine- and lysine-specific cysteine protease activities, which were mostly soluble, were approx. 90% lower than that of the parent strain. Expression of the rgpA, rgpB, and kgp protease genes was the same in **P. gingivalis** FLL92 as in the wild-type strain. In contrast to the parent strain, **P. gingivalis** FLL92 showed increased autoaggregation in addition to a significant reduction in

hemagglutinating and hemolysin activities. In in vivo expts. using a mouse model, **P. gingivalis** FLL92 was dramatically less virulent than the parent strain. A mol. survey of this mutant and the parent strain using all known **P. gingivalis** insertion sequence elements as probes suggested that no intragenomic changes due to the movement of these elements have occurred in **P. gingivalis** FLL92. Taken together, these results suggest that the **recA** downstream gene, designated vimA (virulence-modulating gene), plays an important role in virulence modulation in **P. gingivalis** W83, possibly representing a novel posttranscriptional or translational regulation of virulence factors in **P. gingivalis**.

REFERENCE COUNT: 66 THERE ARE 66 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 6 OF 9 HCAPLUS COPYRIGHT 2004 ACS on STN
ED Entered STN: 29 Nov 2000
ACCESSION NUMBER: 2000:833810 HCAPLUS
DOCUMENT NUMBER: 134:173773
TITLE: The **recA** gene in **Porphyromonas gingivalis**: Expression and regulation
AUTHOR(S): Liu, Yi
CORPORATE SOURCE: Loma Linda Univ., USA
SOURCE: (2000) 164 pp. Avail.: UMI, Order No. DA9964931 From: Diss. Abstr. Int., B 2000, 61(3), 1195
DOCUMENT TYPE: Dissertation
LANGUAGE: English
AB Unavailable

L2 ANSWER 7 OF 9 HCAPLUS COPYRIGHT 2004 ACS on STN
ED Entered STN: 25 Feb 2000
ACCESSION NUMBER: 2000:133552 HCAPLUS
DOCUMENT NUMBER: 132:165121
TITLE: Non-virulent **Porphyromonas gingivalis** mutant
INVENTOR(S): Fletcher, Hansel M.
PATENT ASSIGNEE(S): Loma Linda University, USA
SOURCE: PCT Int. Appl., 31 pp. CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000009156	A1	20000224	WO 1999-US18197	19990811

W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE,

DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

US 6254863	B1	20010703	US 1998-133089	19980812
CA 2340070	AA	20000224	CA 1999-2340070	19990811
AU 9956724	A1	20000306	AU 1999-56724	19990811
AU 761114	B2	20030529		
EP 1105156	A1	20010613	EP 1999-943674	19990811
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
US 6585977	B1	20030701	US 2001-762618	20010209
US 2001012512	A1	20010809	US 2001-803766	20010312
US 6586227	B2	20030701		

PRIORITY APPLN. INFO.: US 1998-133089 A 19980812
WO 1999-US18197 W 19990811

AB A non-virulent, **recA** defective mutant of **Porphyromonas gingivalis** is disclosed which is deposited at ATCC under accession number 202109. Also disclosed is a method of decreasing the growth rate or reproduction rate of **Porphyromonas gingivalis** in a mammal comprising the step of administering to the mammal at least one dose of **Porphyromonas gingivalis** according to the present invention. Further, a method of preventing or treating a **Porphyromonas gingivalis** infection such as periodontitis in a mammal comprising the step of administering to the mammal at least one immunizing dose of **Porphyromonas gingivalis** according to the present invention is described. Also, a pharmaceutical composition comprising a non-virulent, **recA** defective mutant of **Porphyromonas gingivalis** is claimed.

REFERENCE COUNT: 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 8 OF 9 HCAPLUS COPYRIGHT 2004 ACS on STN
ED Entered STN: 09 Feb 2000
ACCESSION NUMBER: 2000:90228 HCAPLUS
DOCUMENT NUMBER: 132:276519
TITLE: Unaltered expression of the major protease genes in a non-virulent **recA**-defective mutant of **Porphyromonas gingivalis** W83
AUTHOR(S): Abaibou, H.; Ma, Q.; Olango, G. J.; Potempa, J.; Travis, J.; Fletcher, H. M.
CORPORATE SOURCE: Department of Microbiology and Molecular Genetics, Loma Linda University, Loma Linda, CA, 92350, USA
SOURCE: Oral Microbiology and Immunology (2000), 15(1), 40-47
CODEN: OMIMEE; ISSN: 0902-0055
PUBLISHER: Munksgaard International Publishers Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB **Porphyromonas gingivalis** FLL32, a **recA** mutant, was isolated during construction of a **recA** defective mutant of **P. gingivalis** W83 by allelic exchange mutagenesis. In contrast to W83 and FLL33, the typical

recA- mutant previously reported, FLL32 was non-pigmented, lacked β-hemolytic activity on blood agar and produced significantly less proteolytic activity. The proteolytic activity in FLL32 was mostly soluble Expression of the rgpA, rgpB and kgp protease genes was unaltered in FLL32 when compared to FLL33 and the wild-type strain. FLL32 exhibited reduced virulence in a murine model and partially protected the animals immunized with that strain against a subsequent lethal challenge by the wild-type strain. These results indicate that the reduced level of proteolytic activity in FLL32 may be due to a defect in the processing of the proteases. Further, immunization with a non-virulent **recA** defective mutant of **P. gingivalis** can partially protect against a lethal wild-type challenge. The results from this study suggest that the **recA** locus may be involved in expression and regulation of proteolytic activity in **P. gingivalis**.

REFERENCE COUNT: 34 THERE ARE 34 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 9 OF 9 HCAPLUS COPYRIGHT 2004 ACS on STN
ED Entered STN: 20 Nov 1997
ACCESSION NUMBER: 1997:730382 HCAPLUS
DOCUMENT NUMBER: 128:58048
TITLE: Nucleotide sequence of the **Porphyromonas gingivalis** W83 **recA** homolog and construction of a **recA**-deficient mutant
AUTHOR(S): Fletcher, Hansel M.; Morgan, Roderick M.; Macrina, Francis L.
CORPORATE SOURCE: Dep. Microbiology & Molecular Genetics, Loma Linda Univ., Loma Linda, CA, 92350, USA
SOURCE: Infection and Immunity (1997), 65(11), 4592-4597
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Degenerate oligonucleotide primers were used in PCR to amplify a region of the **recA** homolog from **Porphyromonas gingivalis** W83. The resulting PCR fragment was used as a probe to identify a recombinant λ DASH phage (L10) carrying the **P. gingivalis recA** homolog. The **recA** homolog was localized to a 2.1-kb BamHI fragment. The nucleotide sequence of this 2.1-kb fragment was determined, and a 1.02-kb open reading frame (341 amino acids) was detected. The predicted amino acid sequence was strikingly similar (90% identical residues) to the **RecA** protein from Bacteroides fragilis. No SOS box, characteristic of LexA-regulated promoters, was found in the 5' upstream region of the **P. gingivalis** **recA** homolog. In both Me methanesulfonate and UV survival expts. the **recA** homolog from **P. gingivalis** complemented the **recA** mutation of Escherichia coli HB101. The cloned **P. gingivalis** **recA** gene was insertionally inactivated with the ermF-ermAM antibiotic resistance cassette to create a **recA**-deficient mutant (FLL33) by allelic exchange. The **recA**-deficient

mutant was significantly more sensitive to UV irradiation than the wild-type strain, W83. W83 and FLL33 showed the same levels of virulence in in vivo expts. using a mouse model. These results suggest that the **recA** gene in **P. gingivalis** W83 plays the expected role of repairing DNA damage caused by UV irradiation However, inactivation of this gene did not alter the virulence of **P. gingivalis** in the mouse model.

REFERENCE COUNT: 39 THERE ARE 39 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

(FILE 'MEDLINE, BIOSIS, EMBASE, WPIDS, JICST-EPLUS, JAPIO, PHIC, PHIN, TOXCENTER, DISSABS, PASCAL, FEDRIP' ENTERED AT 08:55:56 ON 21 MAY 2004)

L3 32 S L2

L4 14 DUP REM L3 (18 DUPLICATES REMOVED)

L4 ANSWER 1 OF 14 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN

ACCESSION NUMBER: 2003:354507 BIOSIS
DOCUMENT NUMBER: PREV200300354507
TITLE: Non-virulent **Porphyromonas gingivalis** mutant.
AUTHOR(S): Fletcher, Hansel M. [Inventor, Reprint Author]
CORPORATE SOURCE: Loma Linda, CA, USA
ASSIGNEE: Loma Linda University
PATENT INFORMATION: US 6585977 July 01, 2003
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (July 1 2003) Vol. 1272, No. 1. http://www.uspto.gov/web/menu/patdata.html. e-file.
ISSN: 0098-1133 (ISSN print).
DOCUMENT TYPE: Patent
LANGUAGE: English
ENTRY DATE: Entered STN: 30 Jul 2003
Last Updated on STN: 30 Jul 2003

AB A non-virulent, **recA** defective mutant of **Porphyromonas gingivalis**. The **Porphyromonas gingivalis** strain which is deposited at ATCC under accession number 202109. Also a method of decreasing the growth rate or reproduction rate of **Porphyromonas gingivalis** in a mammal comprising the step of administering to the mammal at least one dose of **Porphyromonas gingivalis** according to the present invention. Further, a method of preventing or treating a **Porphyromonas gingivalis** infection such as periodontitis in a mammal comprising the step of administering to the mammal at least one dose of **Porphyromonas gingivalis** according to the present invention. Further, a method of preventing or treating a **Porphyromonas gingivalis** infection such as periodonitis in a mammal comprising the step of administering to the mammal at least one dose of **Porphyromonas gingivalis** according to the present invention. Also, a pharmaceutical composition comprising a non-virulent, **recA** defective mutant of **Porphyromonas gingivalis**.

L4 ANSWER 2 OF 14 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 2003410671 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12949166
TITLE: Multilocus sequence analysis of **Porphyromonas gingivalis** indicates frequent recombination.
AUTHOR: Koehler Andreas; Karch Helge; Beikler Thomas; Flemmig Thomas F; Suerbaum Sebastian; Schmidt Herbert
CORPORATE SOURCE: Institut fur Hygiene und Mikrobiologie der Bayerischen Julius-Maximilians-Universitat, 97080 Wurzburg, Germany.
SOURCE: Microbiology (Reading, England), (2003 Sep) 149 (Pt 9) 2407-15.
Journal code: 9430468. ISSN: 1350-0872.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AJ555632; GENBANK-AJ555633; GENBANK-AJ555634; GENBANK-AJ555635; GENBANK-AJ555636; GENBANK-AJ555637; GENBANK-AJ555638; GENBANK-AJ555639; GENBANK-AJ555640; GENBANK-AJ555641; GENBANK-AJ555642; GENBANK-AJ555643; GENBANK-AJ555644; GENBANK-AJ555645; GENBANK-AJ555646; GENBANK-AJ555647; GENBANK-AJ555648; GENBANK-AJ555649; GENBANK-AJ555650; GENBANK-AJ555651; GENBANK-AJ555652; GENBANK-AJ555653; GENBANK-AJ555654; GENBANK-AJ555655; GENBANK-AJ555656; GENBANK-AJ555657; GENBANK-AJ555658; GENBANK-AJ555659; GENBANK-AJ555660; GENBANK-AJ555661; GENBANK-AJ555662; GENBANK-AJ555663; GENBANK-AJ555664; GENBANK-AJ555665; GENBANK-AJ555666; GENBANK-AJ555667; GENBANK-AJ555668; GENBANK-AJ555669; GENBANK-AJ555670; GENBANK-AJ555671; GENBANK-AJ555672; GENBANK-AJ555673; GENBANK-AJ555674; GENBANK-AJ555675; GENBANK-AJ555676; GENBANK-AJ555677; GENBANK-AJ555678; GENBANK-AJ555679; GENBANK-AJ555680; GENBANK-AJ555681; GENBANK-AJ555682; GENBANK-AJ555683; GENBANK-AJ555684; GENBANK-AJ555685; GENBANK-AJ555686; GENBANK-AJ555687; GENBANK-AJ555688; GENBANK-AJ555689; GENBANK-AJ555690; GENBANK-AJ555691; GENBANK-AJ555692; GENBANK-AJ555693; GENBANK-AJ555694; GENBANK-AJ555695; GENBANK-AJ555696; GENBANK-AJ555697; GENBANK-AJ555698; GENBANK-AJ555699; GENBANK-AJ555700; GENBANK-AJ555701; GENBANK-AJ555702; GENBANK-AJ555703; GENBANK-AJ555704; GENBANK-AJ555705; GENBANK-AJ555706; GENBANK-AJ555707; GENBANK-AJ555708; GENBANK-AJ555709; GENBANK-AJ555710; GENBANK-AJ555711; GENBANK-AJ555712; GENBANK-AJ555713; GENBANK-AJ555714; GENBANK-AJ555715; GENBANK-AJ555716; GENBANK-AJ555717; GENBANK-AJ555718; GENBANK-AJ555719; GENBANK-AJ555720; GENBANK-AJ555721; GENBANK-AJ555722; GENBANK-AJ555723; GENBANK-AJ555724; GENBANK-AJ555725; GENBANK-AJ555726; GENBANK-AJ555727; GENBANK-AJ555728; GENBANK-AJ555729; GENBANK-AJ555730; GENBANK-AJ555731; GENBANK-AJ555732; GENBANK-AJ555733; GENBANK-AJ555734; GENBANK-AJ555735; GENBANK-AJ555736; GENBANK-AJ555737; GENBANK-AJ555738; GENBANK-AJ555739; GENBANK-AJ555740; GENBANK-AJ555741; GENBANK-AJ555742; GENBANK-AJ555743; GENBANK-AJ555744; GENBANK-AJ555745;

GENBANK-AJ555746; GENBANK-AJ555747; GENBANK-AJ555748;
GENBANK-AJ555749; GENBANK-AJ555750; GENBANK-AJ555751;
GENBANK-AJ555752; GENBANK-AJ555753; GENBANK-AJ555754;
GENBANK-AJ555755; GENBANK-AJ555756; GENBANK-AJ555757;
GENBANK-AJ555758; GENBANK-AJ555759; GENBANK-AJ555760;
GENBANK-AJ555761; GENBANK-AJ555762; GENBANK-AJ555763;
GENBANK-AJ555764; GENBANK-AJ555765; GENBANK-AJ555766;
GENBANK-AJ555767; GENBANK-AJ555768; GENBANK-AJ555769;
GENBANK-AJ555770; GENBANK-AJ555771; GENBANK-AJ555772;
GENBANK-AJ555773; GENBANK-AJ555774; GENBANK-AJ555775;
GENBANK-AJ555776; GENBANK-AJ555777; GENBANK-AJ555778;
GENBANK-AJ555779; GENBANK-AJ555780; GENBANK-AJ555781;
GENBANK-AJ555782; GENBANK-AJ555783; GENBANK-AJ555784;
GENBANK-AJ555785; GENBANK-AJ555786; GENBANK-AJ555787;
GENBANK-AJ555788; GENBANK-AJ555789; GENBANK-AJ555790;
GENBANK-AJ555791; GENBANK-AJ555792; GENBANK-AJ555793;
GENBANK-AJ555794; GENBANK-AJ555795; GENBANK-AJ555796;
GENBANK-AJ555797; GENBANK-AJ555798; GENBANK-AJ555799;
GENBANK-AJ555800; GENBANK-AJ555801; GENBANK-AJ555802;
GENBANK-AJ555803; GENBANK-AJ555804; GENBANK-AJ555805;
GENBANK-AJ555806; GENBANK-AJ555807; GENBANK-AJ555808;
GENBANK-AJ555809; GENBANK-AJ555810; GENBANK-AJ555811;
GENBANK-AJ555812; GENBANK-AJ555813; GENBANK-AJ555814;
GENBANK-AJ555815; GENBANK-AJ555816; GENBANK-AJ555817;
GENBANK-AJ555818; GENBANK-AJ555819; GENBANK-AJ555820;
GENBANK-AJ555821

ENTRY MONTH: 200403
ENTRY DATE: Entered STN: 20030903
Last Updated on STN: 20040305
Entered Medline: 20040304

AB In this study, the genetic relationship of 19 **Porphyromonas gingivalis** isolates from patients with periodontitis was investigated by multilocus sequence analysis. Internal 400-600 bp DNA fragments of the 10 chromosomal genes ef-tu, ftsQ, hagB, gpdxJ, pepO, mcmA, dnaK, **recA**, pga and nah were amplified by PCR and sequenced. No two isolates were identical at all 10 loci. Phylogenetic analyses indicated a panmictic population structure of **P. gingivalis**. Split decomposition analysis, calculation of homoplasy ratios and analyses of clustered polymorphisms all indicate that recombination plays a major role in creating the genetic heterogeneity of **P. gingivalis**. A standardized index of association of 0.0898 indicates that the **P. gingivalis** genes analysed are close to linkage equilibrium.

L4 ANSWER 3 OF 14 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2001:356355 BIOSIS
DOCUMENT NUMBER: PREV200100356355
TITLE: Non-virulent **Porphyromonas gingivalis** mutant.
AUTHOR(S): Fletcher, Hansel M. [Inventor, Reprint author]
CORPORATE SOURCE: Loma Linda, CA, USA
ASSIGNEE: Loma Linda University, Loma Linda, CA, USA
PATENT INFORMATION: US 6254863 July 03, 2001
SOURCE: Official Gazette of the United States Patent and

Trademark Office Patents, (July 3, 2001) Vol. 1248, No. 1. e-file.
CODEN: OGUPE7. ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English
ENTRY DATE: Entered STN: 2 Aug 2001
Last Updated on STN: 19 Feb 2002

AB A non-virulent, **recA** defective mutant of **Porphyromonas gingivalis**. The **Porphyromonas gingivalis** strain which is deposited at ATCC under accession number 202109. Also a method of decreasing the growth rate or reproduction rate of **Porphyromonas gingivalis** in a mammal comprising the step of administering to the mammal at least one dose of **Porphyromonas gingivalis** according to the present invention. Further, a method of preventing or treating a **Porphyromonas gingivalis** infection such as periodontitis in a mammal comprising the step of administering to the mammal at least one dose of **Porphyromonas gingivalis** according to the present invention. Also, a pharmaceutical composition comprising a non-virulent, **recA** defective mutant of **Porphyromonas gingivalis**.

L4 ANSWER 4 OF 14 MEDLINE on STN DUPLICATE 2

ACCESSION NUMBER: 2001088763 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11119521
TITLE: vimA gene downstream of **recA** is involved in virulence modulation in **Porphyromonas gingivalis** W83.
AUTHOR: Abaibou H; Chen Z; Olango G J; Liu Y; Edwards J; Fletcher H M
CORPORATE SOURCE: Department of Microbiology and Molecular Genetics, School of Medicine, Loma Linda University, Loma Linda, California 92350, USA.
CONTRACT NUMBER: DE11864-01A2 (NIDCR)
SOURCE: Infection and immunity, (2001 Jan) 69 (1) 325-35.
Journal code: 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF064682
ENTRY MONTH: 200101
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20010118

AB A 0.9-kb open reading frame encoding a unique 32-kDa protein was identified downstream of the **recA** gene of **Porphyromonas gingivalis**. Reverse transcription-PCR and Northern blot analysis showed that both the **recA** gene and this open reading frame are part of the same transcriptional unit. This cloned fragment was insertionally inactivated using the ermF-ermAM antibiotic resistance cassette to create a defective mutant by allelic exchange. When plated on Brucella blood agar, the mutant strain, designated **P. gingivalis** FLL92, was non-black pigmented and showed

significant reduction in beta-hemolysis compared with the parent strain, **P. gingivalis** W83. Arginine- and lysine-specific cysteine protease activities, which were mostly soluble, were approximately 90% lower than that of the parent strain. Expression of the rgpA, rgpB, and kgp protease genes was the same in **P. gingivalis** FLL92 as in the wild-type strain. In contrast to the parent strain, **P. gingivalis** FLL92 showed increased autoaggregration in addition to a significant reduction in hemagglutinating and hemolysin activities. In in vivo experiments using a mouse model, **P. gingivalis** FLL92 was dramatically less virulent than the parent strain. A molecular survey of this mutant and the parent strain using all known **P. gingivalis** insertion sequence elements as probes suggested that no intragenomic changes due to the movement of these elements have occurred in **P. gingivalis** FLL92. Taken together, these results suggest that the **recA** downstream gene, designated vimA (virulence-modulating gene), plays an important role in virulence modulation in **P. gingivalis** W83, possibly representing a novel posttranscriptional or translational regulation of virulence factors in **P. gingivalis**
.

L4 ANSWER 5 OF 14 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 2001389368 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11442846
TITLE: The **recA** gene in **Porphyromonas gingivalis** is expressed during infection of the murine host.
AUTHOR: Liu Y; Fletcher H M
CORPORATE SOURCE: Department of Microbiology and Molecular Genetics, School of Medicine, Loma Linda University, Loma Linda, California 92350, USA.
CONTRACT NUMBER: DE11864-01A2 (NIDCR)
SOURCE: Oral microbiology and immunology, (2001 Aug) 16 (4) 218-23.
Journal code: 8707451. ISSN: 0902-0055.
PUB. COUNTRY: Denmark
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Dental Journals
ENTRY MONTH: 200111
ENTRY DATE: Entered STN: 20011105
Last Updated on STN: 20011105
Entered Medline: 20011101

AB The **recA** gene in **Porphyromonas gingivalis** is involved in DNA repair. To further elucidate the importance of the **recA** locus in the pathogenesis of **P. gingivalis**, we assessed its ability for expression in an animal host. The promoterless xa-tetA(Q)2 cassette was used in heterodiploid mutants to study **recA** promoter activity during infection. **P. gingivalis** FLL118.1 had the xa-tetA(Q)2 cassette under the control of **recA** promoter whereas **P. gingivalis** FLL119 had the cassette in the opposite orientation. xa encodes a bifunctional xylosidase/arabinosidase enzyme (XA) and the tetA(Q)2

gene product confers tetracycline resistance. Intramuscular infection in a mouse model allowed the recovery of the bacteria from inguinal lymph nodes. Infusion of tetracycline in the animals permitted the enrichment **P. gingivalis** FLL118.1 over the wild-type strain, during a mixed infection. The xylosidase activity of FLL118.1 could be detected on agar plates in the presence of 5-methylumbellifiry-beta-D-xyloside. No such enrichment for xylosidase activity was detected when the mixture of **P. gingivalis** W83 and **P. gingivalis** FLL119 was used to infect the mouse or cultured in vitro. These results indicated that **recA** promoter was transcriptionally active during the infection of the murine host and further support the importance of this locus during the **P. gingivalis** infection process.

L4 ANSWER 6 OF 14 MEDLINE on STN DUPLICATE 4

ACCESSION NUMBER: 2001267137 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11358535
TITLE: Environmental regulation of **recA** gene expression in **Porphyromonas gingivalis**.
AUTHOR: Liu Y; Fletcher H M
CORPORATE SOURCE: Department of Microbiology and Molecular Genetics, School of Medicine, Loma Linda University, Loma Linda, California 92350, USA.
CONTRACT NUMBER: DE11864-01A2 (NIDCR)
SOURCE: Oral microbiology and immunology, (2001 Jun) 16 (3) 136-43.
Journal code: 8707451. ISSN: 0902-0055.
PUB. COUNTRY: Denmark
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Dental Journals
ENTRY MONTH: 200108
ENTRY DATE: Entered STN: 20010813
Last Updated on STN: 20010813
Entered Medline: 20010809

AB The **recA** gene product in **Porphyromonas gingivalis** is involved in DNA repair. Further, disruption of this gene can affect the proteolytic activity and expression of other virulence factors in this organism. Since several known environmental factors can influence virulence gene expression in **P. gingivalis**, we investigated the influence of these signals on the expression of the **recA** gene in this organism. A heterodiploid strain of **P. gingivalis** (designated FLL118) containing a transcriptional fusion of the **recA** promoter region and the promoterless tetracycline-resistant gene [tetA(Q)2] and xylosidase/arabinosidase (xa) gene cassette was constructed. The **recA** promoter activity was assessed by measurement of xylosidase activity in FLL118. The expression remained relatively constant during different growth phases, at different pH levels and in the presence of DNA-damaging agents. In response to hemin limitation and in the presence of calcium there was a moderate increase in **recA** promoter activity. Temperature also affected the expression. The highest level of xylosidase activity was observed in cultures at 32

degrees C with a decline of approximately 46% as growth temperature increased to 41 degrees C. Reverse transcriptase polymerase chain reaction analysis revealed that this regulation may be occurring at the transcriptional level. These results suggest that expression of the **recA** gene in **P. gingivalis** W83 is responsive to several environmental signals but is not regulated by a DNA damage-inducible SOS-like regulatory system.

L4 ANSWER 7 OF 14 DISSABS COPYRIGHT (C) 2004 ProQuest Information and Learning Company; All Rights Reserved on STN

ACCESSION NUMBER: 2000:43855 DISSABS Order Number: AAI9964931
TITLE: The **recA** gene in **Porphyromonas gingivalis**: Expression and regulation
AUTHOR: Liu, Yi [Ph.D.]; Fletcher, Hansel [adviser]
CORPORATE SOURCE: Loma Linda University (0106)
SOURCE: Dissertation Abstracts International, (2000) Vol. 61, No. 3B, p. 1195. Order No.: AAI9964931. 164 pages.
DOCUMENT TYPE: Dissertation
FILE SEGMENT: DAI
LANGUAGE: English

AB The **recA** gene product in **P. gingivalis** is involved in DNA repair. The disruption of this gene can affect the proteolytic activity and expression of other virulence factors in this organism. To further elucidate the importance of the **recA** gene in the pathogenesis of **P. gingivalis**, its in vivo and in vitro expression were investigated. In **P. gingivalis** containing the rgpA::xa-tetA(Q) 2 fusion construct [rgpA encodes for an arginine-specific protease in **P. gingivalis**, xa encodes a bifunctional xylosidase/arabinosidase enzyme and tet(A)Q2 is a tetracycline resistant gene], the expression of the xa gene could be detected both in crude extracts and on agar plates. The xa gene was used as a in this study. To investigate the influence of environmental signals, a heterodiploid strain of **P. gingivalis** containing a transcriptional fusion of the **recA** promoter region and the promoterless xa-tetA(Q) 2 cassette was constructed. The **recA** promoter activity was assessed by measurement of xylosidase activity. The expression remained relatively constant in the presence of DNA damaging agents, indicating the lack of a DNA-damage inducible SOS-like regulatory system. In response to hemin limitation and the presence of calcium there was a significant increase in **recA** promoter activity. As temperature increased, there was decreased expression of this gene, decreased proteolytic activity and a change in its distribution. The coordinate regulation of the **recA** gene with proteolytic activities may be considered an important survival strategy for this organism. In a mouse model, intramuscular infection allowed the recovery of the bacteria from inguinal lymph nodes. During a mixed infection with **P. gingivalis** W83 and FLL118.1, which contains the xa-tetA(Q)2 cassette under the control of **recA** promoter, the expression of tetracycline resistance permitted the enrichment of FLL118.1 over W83. No such enrichment was detected when a mixture of W83 and FLL119, which contains the cassette in the opposite orientation to the **recA** promoter, was used to infect the mice. These results indicated that the **recA** promoter was transcriptionally

active during infection of the murine host.

L4 ANSWER 8 OF 14 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
ACCESSION NUMBER: 2000-224178 [19] WPIDS
DOC. NO. CPI: C2000-068359
TITLE: New non-virulent mutant of **Porphyromonas gingivalis**, useful for preventing or treating a **P.gingivalis** infection e.g. periodontitis in mammals, comprises a defect in the **recA** gene.
DERWENT CLASS: B04
INVENTOR(S): FLETCHER, H M
PATENT ASSIGNEE(S): (UYLO-N) UNIV LOMA LINDA; (FLET-I) FLETCHER H M
COUNTRY COUNT: 89
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2000009156	A1	20000224	(200019)*	EN	31
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ UG ZW					
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZA ZW					
AU 9956724	A	20000306	(200030)		
EP 1105156	A1	20010613	(200134)	EN	
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI					
US 6254863	B1	20010703	(200140)		
US 2001012512	A1	20010809	(200147)		
US 6585977	B1	20030701	(200345)		
US 6586227	B2	20030701	(200345)		
AU 761114	B	20030529	(200346)		
CA 2340070	C	20031111	(200377)	EN	

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2000009156	A1	WO 1999-US18197	19990811
AU 9956724	A	AU 1999-56724	19990811
EP 1105156	A1	EP 1999-943674	19990811
		WO 1999-US18197	19990811
US 6254863	B1	US 1998-133089	19980812
US 2001012512	A1 Div ex	US 1998-133089	19980812
		US 2001-803766	20010312
US 6585977	B1 Cont of	US 1998-133089	19980812
		WO 1999-US18197	19990811
		US 2001-762618	20010209
US 6586227	B2 Div ex	US 1998-133089	19980812
		US 2001-803766	20010312
AU 761114	B	AU 1999-56724	19990811
CA 2340070	C	CA 1999-2340070	19990811
		WO 1999-US18197	19990811

Searcher : Shears 571-272-2528

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 9956724	A Based on	WO 2000009156
EP 1105156	A1 Based on	WO 2000009156
US 2001012512	A1 Div ex	US 6254863
US 6585977	B1 Cont of	US 6254863
	Based on	WO 2000009156
US 6586227	B2 Div ex	US 6254863
AU 761114	B Previous Publ.	AU 9956724
	Based on	WO 2000009156
CA 2340070	C Based on	WO 2000009156

PRIORITY APPLN. INFO: US 1998-133089 19980812; US 2001-803766 20010312; US 2001-762618 20010209

AN 2000-224178 [19] WPIDS

AB WO 200009156 A UPAB: 20000419

NOVELTY - A non-virulent, **recA** defective mutant of **Porphyromonas gingivalis** (I), is new.

DETAILED DESCRIPTION - AN INDEPENDENT CLAIM is also included for the **P.gingivalis** strain which is deposited at ATCC under accession number 202109 (II).

ACTIVITY - Antibacterial.

MECHANISM OF ACTION - Vaccine; **recA** plays an important role in DNA repair after e.g. ultraviolet irradiationdamage.

USE - (I) and/or (II) are useful for treating **P. gingivalis** infection e.g. periodontitis, by decreasing the growth or reproduction rate of **P.gingivalis** in a mammal, preferably a human (claimed). (I) and/or (II) may also be used for preventing **P.gingivalis** infection e.g. periodontitis.

ADVANTAGE - (I) provides an effective prevention and treatment for periodontitis. Furthermore, (I) can be used as a host genetic background to determine the specific roles, interactions, relative importance and regulation of the potential virulence factors produced by wild-type **P.gingivalis**.

Dwg.0/9

L4 ANSWER 9 OF 14 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN

ACCESSION NUMBER: 2000:370895 BIOSIS

DOCUMENT NUMBER: PREV200000370895

TITLE: Environmental regulation of **recA** gene expression in **Porphyromonas gingivalis** W83.

AUTHOR(S): Liu, Y. [Reprint author]; Fletcher, H. M. [Reprint author]

CORPORATE SOURCE: Loma Linda University, Loma Linda, CA, USA

SOURCE: Abstracts of the General Meeting of the American Society for Microbiology, (2000) Vol. 100, pp. 98. print.
Meeting Info.: 100th General Meeting of the American Society for Microbiology. Los Angeles, California,

USA. May 21-25, 2000. American Society for Microbiology.
ISSN: 1060-2011.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 30 Aug 2000
Last Updated on STN: 8 Jan 2002

L4 ANSWER 10 OF 14 MEDLINE on STN DUPLICATE 5
ACCESSION NUMBER: 2001118199 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11155163
TITLE: Unaltered expression of the major protease genes in a non-virulent **recA**-defective mutant of **Porphyromonas gingivalis** W83.
AUTHOR: Abaibou H; Ma Q; Olango G J; Potempa J; Travis J; Fletcher H M
CORPORATE SOURCE: Department of Microbiology and Molecular Genetics, Loma Linda University, Loma Linda, California 92350, USA.
CONTRACT NUMBER: DE 09761 (NIDCR)
R03 DE 11864-01A2 (NIDCR)
SOURCE: Oral microbiology and immunology, (2000 Feb) 15 (1) 40-7.
Journal code: 8707451. ISSN: 0902-0055.
PUB. COUNTRY: Denmark
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Dental Journals
ENTRY MONTH: 200102
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20010215

AB **Porphyromonas gingivalis** FLL32, a **recA** mutant, was isolated during construction of a **recA** defective mutant of **P. gingivalis** W83 by allelic exchange mutagenesis. In contrast to W83 and FLL33, the typical **recA**- mutant previously reported, FLL32 was non-pigmented, lacked beta-hemolytic activity on blood agar and produced significantly less proteolytic activity. The proteolytic activity in FLL32 was mostly soluble. Expression of the rgpA, rgpB and kgp protease genes was unaltered in FLL32 when compared to FLL33 and the wild-type strain. FLL32 exhibited reduced virulence in a murine model and partially protected the animals immunized with that strain against a subsequent lethal challenge by the wild-type strain. These results indicate that the reduced level of proteolytic activity in FLL32 may be due to a defect in the processing of the proteases. Further, immunization with a non-virulent **recA** defective mutant of **P. gingivalis** can partially protect against a lethal wild-type challenge. The results from this study suggest that the **recA** locus may be involved in expression and regulation of proteolytic activity in **P. gingivalis**.

L4 ANSWER 11 OF 14 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN

ACCESSION NUMBER: 1999:324681 BIOSIS
DOCUMENT NUMBER: PREV199900324681
TITLE: Involvement of the **recA** locus in autoaggregation in **Porphyromonas gingivalis** W83.
AUTHOR(S): Abaibou, H. [Reprint author]; Chen, Z. [Reprint author]; Liu, Y. [Reprint author]; Edwards, J. [Reprint author]; Jhuma, Z. [Reprint author]; Fletcher, H. M. [Reprint author]
CORPORATE SOURCE: Loma Linda University, Loma Linda, CA, USA
SOURCE: Abstracts of the General Meeting of the American Society for Microbiology, (1999) Vol. 99, pp. 49. print.
Meeting Info.: 99th General Meeting of the American Society for Microbiology. Chicago, Illinois, USA. May 30-June 3, 1999. American Society for Microbiology.
ISSN: 1060-2011.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
Conference; (Meeting Poster)
LANGUAGE: English
ENTRY DATE: Entered STN: 24 Aug 1999
Last Updated on STN: 24 Aug 1999

L4 ANSWER 12 OF 14 MEDLINE on STN DUPLICATE 6
ACCESSION NUMBER: 1998013087 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9353038
TITLE: Nucleotide sequence of the **Porphyromonas gingivalis** W83 **recA** homolog and construction of a **recA**-deficient mutant.
AUTHOR: Fletcher H M; Morgan R M; Macrina F L
CORPORATE SOURCE: Department of Microbiology and Molecular Genetics, Loma Linda University, California 92350, USA.. HFLETCHER@CCMAIL.LLU.EDU
CONTRACT NUMBER: P50 DE10703 (NIDCR)
R01 DE04224 (NIDCR)
SOURCE: Infection and immunity, (1997 Nov) 65 (11) 4592-7.
Journal code: 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF064682
ENTRY MONTH: 199711
ENTRY DATE: Entered STN: 19971224
Last Updated on STN: 20000303
Entered Medline: 19971113

AB Degenerate oligonucleotide primers were used in PCR to amplify a region of the **recA** homolog from **Porphyromonas gingivalis** W83. The resulting PCR fragment was used as a probe to identify a recombinant lambda DASH phage (L10) carrying the **P. gingivalis recA** homolog. The **recA** homolog was localized to a 2.1-kb BamHI fragment. The nucleotide sequence of this 2.1-kb fragment was determined, and a 1.02-kb open reading frame (341 amino acids) was detected. The predicted amino acid sequence was strikingly similar (90% identical

residues) to the **RecA** protein from Bacteroides fragilis. No SOS box, characteristic of LexA-regulated promoters, was found in the 5' upstream region of the **P. gingivalis** **recA** homolog. In both methyl methanesulfonate and UV survival experiments the **recA** homolog from **P. gingivalis** complemented the **recA** mutation of Escherichia coli HB101. The cloned **P. gingivalis** **recA** gene was insertionally inactivated with the ermF-ermAM antibiotic resistance cassette to create a **recA**-deficient mutant (FLL33) by allelic exchange. The **recA**-deficient mutant was significantly more sensitive to UV irradiation than the wild-type strain, W83. W83 and FLL33 showed the same level of virulence in in vivo experiments using a mouse model. These results suggest that the **recA** gene in **P. gingivalis** W83 plays the expected role of repairing DNA damage caused by UV irradiation. However, inactivation of this gene did not alter the virulence of **P. gingivalis** in the mouse model.

L4 ANSWER 13 OF 14 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN

ACCESSION NUMBER: 1997:282157 BIOSIS
DOCUMENT NUMBER: PREV199799581360
TITLE: Virulence of **recA**-defective mutants of **Porphyromonas gingivalis** W83.
AUTHOR(S): Fletcher, H. M.
CORPORATE SOURCE: Loma Linda Univ., Loma Linda, CA, USA
SOURCE: Abstracts of the General Meeting of the American Society for Microbiology, (1997) Vol. 97, No. 0; pp. 101.
Meeting Info.: 97th General Meeting of the American Society for Microbiology. Miami Beach, Florida, USA. May 4-8, 1997.
ISSN: 1060-2011.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
Conference; (Meeting Poster)
LANGUAGE: English
ENTRY DATE: Entered STN: 3 Jul 1997
Last Updated on STN: 3 Jul 1997

L4 ANSWER 14 OF 14 FEDRIP COPYRIGHT 2004 NTIS on STN

ACCESSION NUMBER: 2004:164718 FEDRIP
NUMBER OF REPORT: CRISP 3R01DE13664-02S1
RESEARCH TITLE: Studies on virulence regulation in Porphyromonas
STAFF: Principal Investigator: FLETCHER, HANSEL M; HFLETCHER@SOM.LLU.EDU, LOMA LINDA UNIVERSITY, SCHOOL OF MEDICINE
PERFORMING ORGN: LOMA LINDA UNIVERSITY, LOMA LINDA, CALIFORNIA
SUPPORTING ORGN: Supported By: NATIONAL INSTITUTE OF DENTAL & CRANIOFACIAL RESEARCH
PROJECT START DATE: 2004 (/01/02)
FISCAL YEAR: 2003
ESTD COMPLETION DATE: 2002 (/28/06)
FUNDING: Supplement (Type 3)

FILE SEGMENT: National Institutes of Health

SUM **Porphyromonas gingivalis**, a black-pigmented, gram- negative anaerobe, is widely implicated as an important etiological agent of periodontal disease. This bacterium expresses several potential virulence factors (e.g., capsule, LPS, fimbriae, membrane vesicles, and hydrolytic enzymes) that may contribute to its pathogenicity. Another virulence factor, the **recA** gene, confers resistance to the oxidative stress environment of the inflammatory periodontal pocket. The **recA** gene product is a key protein in DNA repair that protects **P. gingivalis** from DNA damage induced by bactericidal reactive oxygen derivatives generated in the periodontal pocket by neutrophils and transient air exposure. Our laboratory has identified two genes, vimA and bcp, that may be part of the **recA** transcription unit and may also function in virulence. Further, the vimA-mediated virulence modulation in **P. gingivalis**, may represent a novel posttranscriptional regulation of virulence factors in this organism. Because the BCP homologue may have peroxidase function, and gingipains are involved in heme accumulation which can inactivate H2O2, it might be considered an important strategy for the organism to coordinate its oxidative stress and proteolytic activities. This importance is further supported by observation that the **recA** locus promoter is active during infection of the murine host. Moreover, the promoter activity is affected by temperature, iron and calcium which are factors known to coordinately regulate the expression of other bacterial virulence genes. Our observations, taken together, may suggest an important role for the complex **recA** locus in the survival and virulence of **P. gingivalis**. It is our hypothesis that the bcp-**recA**-vimA transcriptional unit is important for virulence and protection against oxidative stress. Our overall objective is to elucidate the molecular mechanism(s) for the vimA-mediated virulence regulation and examine the relative importance of the bcp-**recA**-vimA operon in oxidative stress resistance in **P. gingivalis**. Specific aims for the proposed research are: 1) To characterize the bcp-**recA**-vimA transcriptional unit in **P. gingivalis** W83. This will include: a) mapping the transcription initiation site; b) verifying the promoter sequence upstream of the primary start site; c) evaluating the effect of the bcp gene on the function on the **recA** and vimA genes; 2) To examine the functional significance of the vimA mutation on protease activation in **P. gingivalis** W83; and 3) To evaluate the importance of the bcp-**recA** -vimA transcriptional unit in oxidative stress protection.

(FILE 'MEDLINE' ENTERED AT 08:57:31 ON 21 MAY 2004)

L5	2071	SEA FILE=MEDLINE ABB=ON	PLU=ON	"PORPHYROMONAS GINGIVALIS"/CT	
L6	64713	SEA FILE=MEDLINE ABB=ON	PLU=ON	"BACTERIAL PROTEINS"/CT	
L7	66981	SEA FILE=MEDLINE ABB=ON	PLU=ON	"DNA-BINDING PROTEINS"/CT	
L8	2223	SEA FILE=MEDLINE ABB=ON	PLU=ON	(D8.586.277.656.850 OR D12.776.97.780)/CT	← RecA proteins
L10	171982	SEA FILE=MEDLINE ABB=ON	PLU=ON	MUTATION/CT	

Searcher : Shears 571-272-2528

L11 17220 SEA FILE=MEDLINE ABB=ON PLU=ON MUTAGENESIS/CT
L12 46341 SEA FILE=MEDLINE ABB=ON PLU=ON "POLYMORPHISM (GENETICS) "/CT
L15 2223 SEA FILE=MEDLINE ABB=ON PLU=ON "REC A PROTEIN"/CT
L16 241 SEA FILE=MEDLINE ABB=ON PLU=ON L5 AND (L6 OR L7 OR L8 OR L15)
L17 25 SEA FILE=MEDLINE ABB=ON PLU=ON L16 AND (L10 OR L11 OR L12)

L17 ANSWER 1 OF 25 MEDLINE on STN
ACCESSION NUMBER: 2003549236 MEDLINE
DOCUMENT NUMBER: PubMed ID: 14622347
TITLE: Construction of a pepO gene-deficient mutant of Porphyromonas gingivalis: potential role of endopeptidase O in the invasion of host cells.
AUTHOR: Ansai T; Yu W; Urnowey S; Barik S; Takehara T
CORPORATE SOURCE: Department of Preventive Dentistry, Kyushu Dental College, Manazuru, Kitakyushu, Japan.
SOURCE: Oral microbiology and immunology, (2003 Dec) 18 (6) 398-400.
Journal code: 8707451. ISSN: 0902-0055.
PUB. COUNTRY: Denmark
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Dental Journals
ENTRY MONTH: 200401
ENTRY DATE: Entered STN: 20031122
Last Updated on STN: 20040117
Entered Medline: 20040116
ED Entered STN: 20031122
Last Updated on STN: 20040117
Entered Medline: 20040116
AB Porphyromonas gingivalis has been isolated from lesions of advanced adult periodontitis, and implicated as a periodontal pathogen. We have previously cloned a novel endopeptidase, designated PepO, from P. gingivalis 381, which exhibited significant homology to human endothelin-converting enzyme (ECE)-1. In order to determine the nature and function of the PepO in the host, a pepO gene-deficient mutant strain was constructed by allelic exchange mutagenesis using the ermF-ermAM cassette. No endopeptidase activity was detected in the pepO-deficient mutant. In addition, adherent HeLa (HEp-2) cells were infected with the mutant and the two wild-type strains for assessment of bacterial invasion by an antibiotic protection assay. The invasion efficiency of the mutant strain was about a quarter of the wild type strains. These results suggest that PepO is involved in the first step, i.e. invasion/lysis of mammalian cell membrane, which affects the kinetics of rate of invasion.

L17 ANSWER 2 OF 25 MEDLINE on STN
ACCESSION NUMBER: 2003366214 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12900027
TITLE: Construction and characterization of a Porphyromonas gingivalis htpG disruption mutant.
AUTHOR: Sweier Domenica G; Combs Allison; Shelburne Charles E; Fenno J Christopher; Lopatin Dennis E
CORPORATE SOURCE: Department of Biologic and Materials Sciences, School

of Dentistry, The University of Michigan, Ann Arbor, MI 48109-1078, USA.. domsw@umich.edu
CONTRACT NUMBER: DE000423 (NIDCR)
DE11117 (NIDCR)
SOURCE: FEMS microbiology letters, (2003 Aug 8) 225 (1) 101-6.
Journal code: 7705721. ISSN: 0378-1097.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200401
ENTRY DATE: Entered STN: 20030806
Last Updated on STN: 20040106
Entered Medline: 20040105
ED Entered STN: 20030806
Last Updated on STN: 20040106
Entered Medline: 20040105
AB Our previous reports implicated the Hsp90 homologue (HtpG) of Porphyromonas gingivalis (Pg) in its virulence in periodontal disease. We investigated the role of the HtpG stress protein in the virulence of Pg. This report describes the (i) expression of a recombinant Pg HtpG (rHtpG), (ii) generation and characterization of a polyclonal rabbit anti-Pg rHtpG antiserum, and (iii) construction of a Pg htpG isogenic mutant and evaluation of the growth, adherence and invasion properties compared to the wild-type parental strain. The disruption of the htpG gene did not significantly affect growth, and had no effect on Pg adherence to and invasion of cultured human cells.

L17 ANSWER 3 OF 25 MEDLINE on STN
ACCESSION NUMBER: 2003103943 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12595429
TITLE: Purification, gene cloning, gene expression, and mutants of Dps from the obligate anaerobe Porphyromonas gingivalis.
AUTHOR: Ueshima Junichi; Shoji Mikio; Ratnayake Dinath B; Abe Kihachiro; Yoshida Shinichi; Yamamoto Kenji; Nakayama Koji
CORPORATE SOURCE: Department of Pharmacology, Graduate School of Dental Science, Kyushu University, Fukuoka 812-8582, Japan.
SOURCE: Infection and immunity, (2003 Mar) 71 (3) 1170-8.
Journal code: 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200303
ENTRY DATE: Entered STN: 20030306
Last Updated on STN: 20030321
Entered Medline: 20030320
ED Entered STN: 20030306
Last Updated on STN: 20030321
Entered Medline: 20030320
AB The periodontopathogen Porphyromonas gingivalis is an obligate anaerobe that is devoid of catalase but exhibits a relatively high

degree of resistance to peroxide stress. In the present study, we demonstrate that P. gingivalis contains a Dps homologue that plays an important role in the protection of cells from peroxide stress. The Dps protein isolated from P. gingivalis displayed a ferritin-like spherical polymer consisting of 19-kDa subunits. Molecular cloning and sequencing of the gene encoding this protein revealed that it had a high similarity in nucleotide and amino acid sequences to Dps proteins from other species. The expression of Dps was significantly increased by exposure of P. gingivalis to atmospheric oxygen in an OxyR-dependent manner, indicating that it is regulated by the reactive oxygen species-regulating gene oxyR. The Dps-deficient mutants, including the dps single mutant and the ftn dps double mutant, showed no viability loss upon exposure to atmospheric oxygen for 6 h. In contrast to the wild type, however, these mutants exhibited the high susceptibility to hydrogen peroxide, thereby disrupting the viability. On the other hand, no significant difference in sensitivity to mitomycin C and metronidazole was observed between the wild type and the mutants. Furthermore, the dps single mutant, compared with the wild type, showed a lower viability in infected human umbilical vein endothelial cells.

L17 ANSWER 4 OF 25 MEDLINE on STN
ACCESSION NUMBER: 2002629228 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12354210
TITLE: Cloning and expression of a Porphyromonas gingivalis gene for protoporphyrinogen oxidase by complementation of a hemG mutant of Escherichia coli.
AUTHOR: Kusaba A; Ansai T; Akifusa S; Nakahigashi K; Taketani S; Inokuchi H; Takehara T
CORPORATE SOURCE: Department of Preventive Dentistry, Kyushu Dental College, Kokurakita-ku, Kitakyushu 803-8580, Japan.
SOURCE: Oral microbiology and immunology, (2002 Oct) 17 (5) 290-5.
Journal code: 8707451. ISSN: 0902-0055.
PUB. COUNTRY: Denmark
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Dental Journals
ENTRY MONTH: 200301
ENTRY DATE: Entered STN: 20021022
Last Updated on STN: 20030129
Entered Medline: 20030128
ED Entered STN: 20021022
Last Updated on STN: 20030129
Entered Medline: 20030128
AB Porphyromonas gingivalis, a bacterium implicated in periodontal pathogenesis, has a growth requirement for iron protoporphyrin IX. By complementation with a P. gingivalis 381 chromosomal DNA library, we were able to isolate a clone that enhanced the poor growth of a hemG mutant of Escherichia coli. The DNA sequence analysis of this clone revealed three open reading frames (ORFs). ORF3 encoded a protein of 466 amino acids with a calculated molecular weight of 51 695 Da. The deduced amino acid sequence of the ORF3 gene had significant similarity to sequences of protoporphyrinogen oxidase (PPO) from Myxococcus xanthus (30% identical residues). When the

ORF3 gene was overexpressed in E. coli, the extract had much higher PPO activity than a control extract, and this activity was inhibited by acifluorfen, a specific inhibitor of PPO. Thus, ORF3 was named PgHemG. Furthermore, several porphyrin-related genes, including hemD, hemN and hemH, were identified in the data bases on the websites available on-line. We postulated that a porphyrin biosynthetic pathway to heme from preuroporphyrin may be conserved in P. gingivalis.

L17 ANSWER 5 OF 25 MEDLINE on STN
ACCESSION NUMBER: 2002348945 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12055284
TITLE: Role of the Streptococcus gordonii SspB protein in the development of Porphyromonas gingivalis biofilms on streptococcal substrates.
AUTHOR: Lamont Richard J; El-Sabaeny Azza; Park Yoonsuk; Cook Guy S; Costerton J William; Demuth Donald R
CORPORATE SOURCE: Department of Oral Biology, Box 357132, University of Washington, Seattle, WA 98195, USA..
lamon@u.washington.edu
CONTRACT NUMBER: DE12505 (NIDCR)
DE12750 (NIDCR)
DE13061 (NIDCR)
SOURCE: Microbiology (Reading, England), (2002 Jun) 148 (Pt 6) 1627-36.
Journal code: 9430468. ISSN: 1350-0872.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200212
ENTRY DATE: Entered STN: 20020703
Last Updated on STN: 20021218
Entered Medline: 20021213
ED Entered STN: 20020703
Last Updated on STN: 20021218
Entered Medline: 20021213
AB Porphyromonas gingivalis is an aggressive periodontal pathogen that persists in the mixed-species plaque biofilm on tooth surfaces. P. gingivalis cells attach to the plaque commensal Streptococcus gordonii and this coadhesion event leads to the development of P. gingivalis biofilms. Binding of these organisms is multimodal, involving both the P. gingivalis major fimbrial FimA protein and the species-specific interaction of the minor fimbrial Mfa1 protein with the streptococcal SspB protein. This study examined the contribution of the Mfa1-SspB interaction to P. gingivalis biofilm formation. P. gingivalis biofilms readily formed on substrata of S. gordonii DL1 but not on Streptococcus mutans cells which lack a coadhesion-mediating homologue of SspB. An insertional inactivation of the mfa1 gene in P. gingivalis resulted in a phenotype deficient in S. gordonii binding and unable to form biofilms. Furthermore, analysis using recombinant streptococci and enterococci showed that P. gingivalis biofilms formed on Enterococcus faecalis strains expressing SspB or translational fusions of SspB with SpaP (the non-adherent SspB homologue in S. mutans) containing the P. gingivalis adherence domain (SspB adherence region, BAR) of SspB.

In contrast, an isogenic Ssp null mutant of S. gordonii DL1 was unable to support biofilm growth, even though this strain bound to P. gingivalis FimA at levels similar to wild-type S. gordonii DL1. Finally, site-specific mutation of two functional amino acid residues in BAR resulted in SspB polypeptides that did not promote the development of P. gingivalis biofilms. These results suggest that the induction of P. gingivalis biofilms on a streptococcal substrate requires functional SspB-minor fimbriae interactions.

L17 ANSWER 6 OF 25 MEDLINE on STN
ACCESSION NUMBER: 2002292362 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12030974
TITLE: Cytokine production induced by a 67-kDa fimbrial protein from Porphyromonas gingivalis.
AUTHOR: Hamada N; Watanabe K; Arai M; Hiramine H; Umemoto T
CORPORATE SOURCE: Department of Oral Microbiology, Kanagawa Dental College, 82 Inaoka-cho, Yokosuka 238-8580, Japan.
SOURCE: Oral microbiology and immunology, (2002 Jun) 17 (3) 197-200.
Journal code: 8707451. ISSN: 0902-0055.
PUB. COUNTRY: Denmark
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Dental Journals
ENTRY MONTH: 200211
ENTRY DATE: Entered STN: 20020529
Last Updated on STN: 20021211
Entered Medline: 20021106
ED Entered STN: 20020529
Last Updated on STN: 20021211
Entered Medline: 20021106
AB Fimbriae have been reported to play an important role in the adherence of Porphyromonas gingivalis to oral surfaces and possibly in triggering host responses. P. gingivalis ATCC 33277 has two distinctly different fimbriae expressed on the cell surface. The 67-kDa fimbriae differ in size and antigenicity from the earlier reported FimA, a major 41-kDa fimbrial component of P. gingivalis. Expression of the 67-kDa fimbriae on the cell surface of a fimA mutant was investigated by electron microscopy. The 67-kDa fimbrial protein was purified from the fimA mutant by sonication, precipitation, and chromatography on a DEAE Sepharose CL-6B column. The N-terminal amino acid sequence of the 67-kDa fimbrillin was distinct from that of the 41-kDa fimbrillin. Moreover, we have found that the 67-kDa fimbrial protein from P. gingivalis ATCC 33277 induced IL-1alpha, IL-beta, IL-6 and TNFalpha cytokine expression in mouse peritoneal macrophages. These results suggest that P. gingivalis 67-kDa fimbriae may play a part in the inflammatory response during the development of periodontal diseases.

L17 ANSWER 7 OF 25 MEDLINE on STN
ACCESSION NUMBER: 2002070576 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11796628
TITLE: Identification and testing of Porphyromonas gingivalis virulence genes with a pPGIVET system.
AUTHOR: Wu Yi; Lee Seok-Woo; Hillman Jeffrey D; Progulske-Fox Ann

Searcher : Shears 571-272-2528

CORPORATE SOURCE: Department of Oral Biology, College of Dentistry, University of Florida, Gainesville, Florida 32610, USA.
CONTRACT NUMBER: DE04529 (NIDCR)
DE07496 (NIDCR)
SOURCE: Infection and immunity, (2002 Feb) 70 (2) 928-37.
Journal code: 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200202
ENTRY DATE: Entered STN: 20020125
Last Updated on STN: 20020222
Entered Medline: 20020221
ED Entered STN: 20020125
Last Updated on STN: 20020222
Entered Medline: 20020221
AB An in vivo expression technology (IVET) system was designed to identify previously unknown virulence genes of Porphyromonas gingivalis. Fourteen ivi (for in vivo induced) genes that are induced during infection in a mouse abscess model were identified in our study. Of these, seven had homology to genes in the NCBI database, and the rest had no homology to reported DNA sequences. In order to determine virulence-related properties of these genes, three mutant strains, deleted of ivi8 (no homology to genes in the database), ivi10 (homologous to a putative TonB-dependent outer membrane receptor protein), and ivi11 (an immunoreactive 33-kDa antigen PG125 in P. gingivalis), were created. The mutants were tested in a mouse abscess model for alterations in virulence relative to the wild type by a competition assay in BALB/c mice. After 5 days we observed the enrichment of the wild-type strain over mutant strains Deltaivi10 and Deltaivi11, which indicated that mutant strains Deltaivi10 and Deltaivi11 are less able to survive in this model than the wild-type strain, while Deltaivi8 survives as well as the wild-type strain. We propose that knockout of these ivi genes reduced the ability of the mutated P. gingivalis to survive and cause infection compared to the wild-type strain at the site of injection. Also, in separate experiments, groups of mice were challenged with subcutaneous injections of each individual mutant strain (Deltaivi8, Deltaivi10, and Deltaivi11) or with the wild-type strain alone and were then examined to assess their general health status. The results showed that knockout of these ivi genes conferred a reduction in virulence. The ability of the mutants to invade KB cells compared to the wild type was also determined. Interestingly, the CFU counts of the mutant strain Deltaivi10 recovered from KB cells were eight times lower than those of the wild type, indicating that this mutant has a lower capacity for invasion. These results demonstrate that IVET is a powerful tool in discovering virulence genes and the significant role that ivi genes play in the pathogenesis of this species.

L17 ANSWER 8 OF 25 MEDLINE on STN
ACCESSION NUMBER: 2002036173 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11761879
TITLE: Molecular genetic study of pathogenesis of the oral

anaerobic bacterium Porphyromonas gingivalis.
AUTHOR: Nakayama K
CORPORATE SOURCE: Department of Microbiology, Nagasaki University School of Dentistry, Sakamoto 1-7-1, Nagasaki 852-8588.
SOURCE: Nippon saikingaku zasshi. Japanese journal of bacteriology, (2001) 56 (4) 573-85. Ref: 64
Journal code: 2984804R. ISSN: 0021-4930.
PUB. COUNTRY: Japan
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: Japanese
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200112
ENTRY DATE: Entered STN: 20020124
Last Updated on STN: 20020124
Entered Medline: 20011228
ED Entered STN: 20020124
Last Updated on STN: 20020124
Entered Medline: 20011228

L17 ANSWER 9 OF 25 MEDLINE on STN
ACCESSION NUMBER: 2001638346 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11694077
TITLE: Coaggregation of Porphyromonas gingivalis and Prevotella intermedia.
AUTHOR: Kamaguch A; Nakayama K; Ohyama T; Watanabe T; Okamoto M; Baba H
CORPORATE SOURCE: Department of Oral Microbiology, School of Dentistry, Health Sciences University of Hokkaido, Hokkaido 061-0293, Japan.
SOURCE: Microbiology and immunology, (2001) 45 (9) 649-56.
Journal code: 7703966. ISSN: 0385-5600.
PUB. COUNTRY: Japan
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200205
ENTRY DATE: Entered STN: 20011107
Last Updated on STN: 20020517
Entered Medline: 20020516
ED Entered STN: 20011107
Last Updated on STN: 20020517
Entered Medline: 20020516
AB Porphyromonas gingivalis cells coaggregated with Prevotella intermedia cells. The coaggregation was inhibited with L-arginine, L-lysine, Nalpha-p-tosyl-L-lysine chloromethyl ketone, trypsin inhibitor, and leupeptin. Heat- and proteinase K-treated P. gingivalis cells showed no coaggregation with P. intermedia cells, whereas heat and proteinase K treatments of P. intermedia cells did not affect the coaggregation. The vesicles from P. gingivalis culture supernatant aggregated with P. intermedia cells, and this aggregation was also inhibited by addition of L-arginine or L-lysine and by heat treatment of the vesicles. The rgpA rgpB, rgpA kgp, rgpA rgpB kgp, and rgpA kgp hagA mutants of P. gingivalis did not

coaggregate with P. intermedia. On the other hand, the fimA mutant lacking the FimA fimbriae showed coaggregation with P. intermedia as well as the wild type parent. These results strongly imply that a heat-labile and proteinous factor on the cell surface of P gingivalis, most likely the gingipain-adhesin complex, is involved in coaggregation of P. gingivalis and P. intermedia.

L17 ANSWER 10 OF 25 MEDLINE on STN
ACCESSION NUMBER: 2001389368 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11442846
TITLE: The recA gene in Porphyromonas gingivalis is expressed during infection of the murine host.
AUTHOR: Liu Y; Fletcher H M
CORPORATE SOURCE: Department of Microbiology and Molecular Genetics, School of Medicine, Loma Linda University, Loma Linda, California 92350, USA.
CONTRACT NUMBER: DE11864-01A2 (NIDCR)
SOURCE: Oral microbiology and immunology, (2001 Aug) 16 (4) 218-23.
Journal code: 8707451. ISSN: 0902-0055.
PUB. COUNTRY: Denmark
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Dental Journals
ENTRY MONTH: 200111
ENTRY DATE: Entered STN: 20011105
Last Updated on STN: 20011105
Entered Medline: 20011101
ED Entered STN: 20011105
Last Updated on STN: 20011105
Entered Medline: 20011101
AB The recA gene in Porphyromonas gingivalis is involved in DNA repair. To further elucidate the importance of the recA locus in the pathogenesis of P. gingivalis, we assessed its ability for expression in an animal host. The promoterless xa-tetA(Q)2 cassette was used in heterodiploid mutants to study recA promoter activity during infection. P. gingivalis FLL118.1 had the xa-tetA(Q)2 cassette under the control of recA promoter whereas P. gingivalis FLL119 had the cassette in the opposite orientation. xa encodes a bifunctional xylosidase/arabinosidase enzyme (XA) and the tetA(Q)2 gene product confers tetracycline resistance. Intramuscular infection in a mouse model allowed the recovery of the bacteria from inguinal lymph nodes. Infusion of tetracycline in the animals permitted the enrichment P. gingivalis FLL118.1 over the wild-type strain, during a mixed infection. The xylosidase activity of FLL118.1 could be detected on agar plates in the presence of 5-methylumbellifiry-beta-D-xyloside. No such enrichment for xylosidase activity was detected when the mixture of P. gingivalis W83 and P. gingivalis FLL119 was used to infect the mouse or cultured in vitro. These results indicated that recA promoter was transcriptionally active during the infection of the murine host and further support the importance of this locus during the P. gingivalis infection process.

L17 ANSWER 11 OF 25 MEDLINE on STN
ACCESSION NUMBER: 2001122149 MEDLINE

DOCUMENT NUMBER: PubMed ID: 11094282
TITLE: Purification and characterization of a novel secondary fimbrial protein from Porphyromonas gingivalis strain 381.
AUTHOR: Arai M; Hamada N; Umemoto T
CORPORATE SOURCE: Department of Oral Microbiology, Kanagawa Dental College, 82 Inaoka-cho, 238-8580, Yokosuka, Japan.
SOURCE: FEMS microbiology letters, (2000 Dec 1) 193 (1) 75-81.
Journal code: 7705721. ISSN: 0378-1097.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200102
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20010222

ED Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20010222

AB We previously reported the existence of two different kinds of fimbriae expressed by Porphyromonas gingivalis ATCC 33277. In this study, we isolated and characterized a secondary fimbrial protein from strain FPG41, a fimA-inactivated mutant of P. gingivalis 381. FPG41 was constructed by a homologous recombination technique using a mobilizable suicide vector, and failed to express the long fimbriae (41-kDa fimbriae) that were produced on the cell surface of P. gingivalis 381. However, short fimbrial structures were observed on the cell surface of FPG41 by electron microscopy. The fimbrial protein was purified from FPG41 by DEAE-Sepharose CL-6B column chromatography. The secondary fimbrial protein was eluted at 0.15 M NaCl, and the molecular mass of this protein was approximately 53 kDa as estimated by SDS-PAGE. An antibody against the 53-kDa fimbrial protein reacted with the short fimbriae of the FPG41 and the wild-type strain. However, the 41-kDa long fimbriae of the wild-type strain and the 67-kDa fimbriae of ATCC 33277 did not react with the same antibody. Moreover, the N-terminal amino acid sequence of the 53-kDa fimbrial protein showed only 2 of 15 residues that were identical to those of the 41-kDa fimbrial protein. These results show that the properties of the 53-kDa fimbriae are different from those of the 67-kDa fimbriae of ATCC 33277 as well as those of the 41-kDa fimbriae.

L17 ANSWER 12 OF 25 MEDLINE on STN
ACCESSION NUMBER: 2001053020 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11083767
TITLE: Regulation of the Porphyromonas gingivalis fimA (Fimbrillin) gene.
AUTHOR: Xie H; Chung W O; Park Y; Lamont R J
CORPORATE SOURCE: School of Dentistry, Meharry Medical College, Nashville, Tennessee 37208, USA.. hxie@mail.mmc.edu
CONTRACT NUMBER: DE00401 (NIDCR)
DE11111 (NIDCR)
DE12505 (NIDCR)
SOURCE: Infection and immunity, (2000 Dec) 68 (12) 6574-9.

Journal code: 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200012
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20001213
ED Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20001213
AB In common with many bacterial virulence genes, the fimbrillin (fimA) gene of Porphyromonas gingivalis is modulated in response to environmental fluctuation. The trans-acting components that comprise the regulatory system for transcriptional activity of the fimA gene in P. gingivalis were investigated. Three major proteins were found to bind to the upstream region of the fimA promoter. One of these proteins was fimbrillin itself, and the other two were a major arginine protease (Rgp) and lysine protease (Kgp). Production of these proteins was necessary for maximal fimA transcription. An exogenous fimA promoter-lacZ reporter was inactive when introduced into a strain of P. gingivalis carrying a mutation in the indigenous fimA gene. Furthermore, fimA mRNA levels were significantly decreased in rgp and kgp mutant strains. These data indicate that P. gingivalis has evolved multiple levels of control of fimbrial gene expression to enhance its survival in hostile environments.

L17 ANSWER 13 OF 25 MEDLINE on STN
ACCESSION NUMBER: 2001015459 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10811640
TITLE: Human lactoferrin binds and removes the hemoglobin receptor protein of the periodontopathogen Porphyromonas gingivalis.
AUTHOR: Shi Y; Kong W; Nakayama K
CORPORATE SOURCE: Department of Microbiology, Faculty of Dentistry, Kyushu University, Fukuoka 812-8582, Japan.
SOURCE: Journal of biological chemistry, (2000 Sep 29) 275 (39) 30002-8.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200010
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20001027
ED Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20001027
AB Porphyromonas gingivalis possesses a hemoglobin receptor (HbR) protein on the cell surface as one of the major components of the hemoglobin utilization system in this periodontopathogenic bacterium. HbR is intragenically encoded by the genes of an arginine-specific cysteine proteinase (rgpA), lysine-specific

cysteine proteinase (kgp), and a hemagglutinin (hagA). Here, we have demonstrated that human lactoferrin as well as hemoglobin have the abilities to bind purified HbR and the cell surface of P. gingivalis through HbR. The interaction of lactoferrin with HbR led to the release of HbR from the cell surface of P. gingivalis. This lactoferrin-mediated HbR release was inhibited by the cysteine proteinase inhibitors effective to the cysteine proteinases of P. gingivalis. P. gingivalis could not utilize lactoferrin for its growth as an iron source and, in contrast, lactoferrin inhibited the growth of the bacterium in a rich medium containing hemoglobin as the sole iron source. Lactoferricin B, a 25-amino acid-long peptide located at the N-lobe of bovine lactoferrin, caused the same effects on P. gingivalis cells as human lactoferrin, indicating that the effects of lactoferrin might be attributable to the lactoferricin region. These results suggest that lactoferrin has a bacteriostatic action on P. gingivalis by binding HbR, removing it from the cell surface, and consequently disrupting the iron uptake system from hemoglobin.

L17 ANSWER 14 OF 25 MEDLINE on STN
ACCESSION NUMBER: 2000414672 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10832973
TITLE: Identification of a two-component signal transduction system involved in fimbriation of Porphyromonas gingivalis.
AUTHOR: Hayashi J; Nishikawa K; Hirano R; Noguchi T; Yoshimura F
CORPORATE SOURCE: Department of Periodontology, School of Dentistry, Aichi-Gakuin University, Nagoya, Aichi, Japan.
SOURCE: Microbiology and immunology, (2000) 44 (4) 279-82. Journal code: 7703966. ISSN: 0385-5600.
PUB. COUNTRY: Japan
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AB025360
ENTRY MONTH: 200008
ENTRY DATE: Entered STN: 20000907
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Entered Medline: 20000831

ED Entered STN: 20000907
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Entered Medline: 20000831

AB Porphyromonas gingivalis, a periodontopathogen, is an oral anaerobic gram-negative bacterium with numerous fimbriae on the cell surface. Fimbriae have been considered to be an important virulence factor in this organism. We analyzed the genomic DNA of transposon-induced, fimbria-deficient mutants derived from ATCC 33277 and found that seven independent mutants had transposon insertions within the same restriction fragment. Cloning and sequencing of the disrupted region from one of the mutants revealed two adjacent open reading frames (ORFs) which seemed to encode a two-component signal transduction system. We also found that six of the mutants had insertions in a gene, fimS, a homologue of the genes encoding sensor kinase, and that the insertion in the remaining one disrupted the gene immediately downstream, fimR, a homologue of the response

regulator genes in other bacteria. These findings suggest that this two-component regulatory system is involved in fimbriation of P. gingivalis.

L17 ANSWER 15 OF 25 MEDLINE on STN
ACCESSION NUMBER: 2000049835 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10585140
TITLE: Characterization of an outer membrane protein gene, pgmA, and its gene product from Porphyromonas gingivalis.
AUTHOR: Hongo H; Osano E; Ozeki M; Onoe T; Watanabe K; Honda O; Tani H; Nakamura H; Yoshimura F
CORPORATE SOURCE: Department of Preventive Dentistry, School of Dentistry, Hokkaido University, Sapporo, Japan.
SOURCE: Microbiology and immunology, (1999) 43 (10) 937-46. Journal code: 7703966. ISSN: 0385-5600.
PUB. COUNTRY: Japan
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AB004560
ENTRY MONTH: 200002
ENTRY DATE: Entered STN: 20000309
Last Updated on STN: 20000309
Entered Medline: 20000222
ED Entered STN: 20000309
Last Updated on STN: 20000309
Entered Medline: 20000222
AB A gene upstream from fimA, the gene encoding fimbrilin, on the chromosome of Porphyromonas gingivalis was sequenced and shown to be the gene encoding an outer membrane protein in this organism based on homology and biochemical analyses. Therefore, the gene (formerly ORF5) was designated pgmA, the P. gingivalis outer membrane protein A gene. The gene product, PgmA, was sensitive to protease, and was detected as a 60-kDa protein from wild-type strains with trichloroacetic acid treatment, which was carried out to destroy intrinsic proteases, and from protease-deficient mutants without this treatment prior to electrophoresis. PgmA was indeed present in the membrane fraction. Its nature was determined to be that of outer membrane proteins in gram-negative bacteria based on attempts at differential extraction of inner membrane proteins with detergents. No evidence has been found thus far from functional analyses that this protein is related to fimbrial morphogenesis and functions or to serum resistance of this organism.

L17 ANSWER 16 OF 25 MEDLINE on STN
ACCESSION NUMBER: 1998455816 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9782496
TITLE: Altered expression and modification of proteases from an avirulent mutant of Porphyromonas gingivalis W50 (W50/BE1).
AUTHOR: Collinson L M; Rangarajan M; Curtis M A
CORPORATE SOURCE: Department of Oral Microbiology, St Bartholomew's, London, UK.
SOURCE: Microbiology (Reading, England), (1998 Sep) 144 (Pt 9) 2487-96.

Journal code: 9430468. ISSN: 1350-0872.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199901
ENTRY DATE: Entered STN: 19990115
Last Updated on STN: 20000303
Entered Medline: 19990104

ED Entered STN: 19990115
Last Updated on STN: 20000303
Entered Medline: 19990104

AB Proteases of Porphyromonas gingivalis are considered to be important factors in the virulence of this organism. A non-pigmenting mutant of P. gingivalis W50 (W50/BE1) has been shown to be less virulent in animal models and to produce significantly less Arg-specific protease activity than the parent strain. Three proteases are present in the culture supernatant of P. gingivalis W50: RI, RIA and RIB. All three proteases are derived from prpR1, which encodes a polypeptide of 1706 amino acids that is organized into distinct domains (pro, alpha, beta and gamma). The aim of the present investigation was to purify and characterize the Arg-specific proteases produced by the avirulent W50/BE1 strain. Significant differences were observed between the proteases of P. gingivalis W50 and W50/BE1. The levels of RI present in the culture supernatant of W50/BE1 were lower than those present in W50, and RIA and RIB were absent. RI from W50/BE1 was composed of three polypeptide chains, unlike the enzyme from W50, which is a heterodimer. The remainder of the Arg-specific protease activity in W50/BE1 was derived from a second gene, prR2, and was present in two fractions, RIIAs/BE (soluble) and RIIAv/BE (vesicle-bound). This activity contained two peptide chains: a approximately 54 kDa chain corresponding to the protease domain and a approximately 26 kDa chain, derived from the propeptide domain of the PrRII precursor. No enzyme with large glycan additions, equivalent to RIB in the vesicle fraction of the wild-type W50, was present. These data indicate that the reduced level of extracellular protease activity in W50/BE1 reflects reduced synthesis and/or export of prpR1 enzymes, which is only partially compensated by synthesis of prR2-derived enzymes, and that all of these proteases undergo altered post-translational modification compared to the parent strain.

L17 ANSWER 17 OF 25 MEDLINE on STN
ACCESSION NUMBER: 1998133982 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9466944
TITLE: Isolation and characterization of transposon-induced mutants of Porphyromonas gingivalis deficient in fimbriation.
AUTHOR: Watanabe-Kato T; Hayashi J I; Terazawa Y; Hoover C I; Nakayama K; Hibi E; Kawakami N; Ikeda T; Nakamura H; Noguchi T; Yoshimura F
CORPORATE SOURCE: Department of Endodontics, School of Dentistry, Aichi-Gakuin University, Nagoya, 464, Japan.
SOURCE: Microbial pathogenesis, (1998 Jan) 24 (1) 25-35. Journal code: 8606191. ISSN: 0882-4010.
PUB. COUNTRY: ENGLAND: United Kingdom

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199803
ENTRY DATE: Entered STN: 19980407
Last Updated on STN: 20000303
Entered Medline: 19980324

ED Entered STN: 19980407
Last Updated on STN: 20000303
Entered Medline: 19980324

AB Fimbriae are considered to be an important virulence factor of Porphyromonas gingivalis. In order to identify genes essential for fimbriation, other than fimA which encodes the major subunit protein of fimbriae, transposon mutagenesis and immunological screening techniques were used to isolate fimbria-deficient mutants. R751::*Omega4, a suicide vector that carries Tn4351, was transferred from Escherichia coli to P. gingivalis by conjugation. Twenty-two independent fimbria-deficient mutants were identified among the resulting transformants. Southern hybridization analysis with pBlue 4351, a transposon-specific probe, and R751 indicated that 45% of the mutants resulted from single transposon insertions and that the remaining 55% of the mutants resulted from cointegration of R751 sequences. Southern hybridization analysis with pUCBg12.1, a probe for the fimA region, indicated that nine of the mutants contained insertions within the 2.5 kb SacI DNA fragment of P. gingivalis that contains fimA, ORF1 (which encodes a 15 kDa protein), and the C-terminal portion of ORF5 (which encodes a 63 kDa protein). Polymerase chain reaction (PCR) analysis and further Southern hybridization analysis indicated that the insertion site(s) for all nine of these mutants was within the fimA gene. Southern hybridization analysis also indicated that the remaining thirteen mutants contained insertions somewhere outside the 10 kb fimA region. Analysis by pulsed field gel electrophoresis (PFGE) revealed that insertions for most of the thirteen mutants mapped to a 300 kb NotI fragment and are located at least approximately 200 kb away from fimA. These results identify genetic loci other than fimA, that are required for fimbriation of P. gingivalis. Future cloning and characterization of these genetic loci should be straightforward since they are now marked by antibiotic resistance genes carried by the transposon.

L17 ANSWER 18 OF 25 MEDLINE on STN
ACCESSION NUMBER: 1998013087 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9353038
TITLE: Nucleotide sequence of the Porphyromonas gingivalis W83 recA homolog and construction of a recA-deficient mutant.
AUTHOR: Fletcher H M; Morgan R M; Macrina F L
CORPORATE SOURCE: Department of Microbiology and Molecular Genetics, Loma Linda University, California 92350, USA.. HFLETCHER@CCMAIL.LLU.EDU
CONTRACT NUMBER: P50 DE10703 (NIDCR)
R01 DE04224 (NIDCR)
SOURCE: Infection and immunity, (1997 Nov) 65 (11) 4592-7.
Journal code: 0246127. ISSN: 0019-9567.

PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF064682
ENTRY MONTH: 199711
ENTRY DATE: Entered STN: 19971224
Last Updated on STN: 20000303
Entered Medline: 19971113

ED Entered STN: 19971224
Last Updated on STN: 20000303
Entered Medline: 19971113

AB Degenerate oligonucleotide primers were used in PCR to amplify a region of the recA homolog from Porphyromonas gingivalis W83. The resulting PCR fragment was used as a probe to identify a recombinant lambda DASH phage (L10) carrying the P. gingivalis recA homolog. The recA homolog was localized to a 2.1-kb BamHI fragment. The nucleotide sequence of this 2.1-kb fragment was determined, and a 1.02-kb open reading frame (341 amino acids) was detected. The predicted amino acid sequence was strikingly similar (90% identical residues) to the RecA protein from Bacteroides fragilis. No SOS box, characteristic of LexA-regulated promoters, was found in the 5' upstream region of the P. gingivalis recA homolog. In both methyl methanesulfonate and UV survival experiments the recA homolog from P. gingivalis complemented the recA mutation of Escherichia coli HB101. The cloned P. gingivalis recA gene was insertionally inactivated with the ermF-ermAM antibiotic resistance cassette to create a recA-deficient mutant (FLL33) by allelic exchange. The recA-deficient mutant was significantly more sensitive to UV irradiation than the wild-type strain, W83. W83 and FLL33 showed the same level of virulence in in vivo experiments using a mouse model. These results suggest that the recA gene in P. gingivalis W83 plays the expected role of repairing DNA damage caused by UV irradiation. However, inactivation of this gene did not alter the virulence of P. gingivalis in the mouse model.

L17 ANSWER 19 OF 25 MEDLINE on STN
ACCESSION NUMBER: 97386416 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9244265
TITLE: The Tla protein of Porphyromonas gingivalis W50: a homolog of the RI protease precursor (PrpRI) is an outer membrane receptor required for growth on low levels of hemin.
AUTHOR: Aduse-Opoku J; Slaney J M; Rangarajan M; Muir J; Young K A; Curtis M A
CORPORATE SOURCE: Department of Oral Microbiology, St. Bartholomew's and the Royal London School of Medicine and Dentistry, Queen Mary and Westfield College, England.. J.Aduse@mds.qmw.ac.uk
SOURCE: Journal of bacteriology, (1997 Aug) 179 (15) 4778-88. Journal code: 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-P27772; GENBANK-U00007; GENBANK-U59691;

GENBANK-V56084; GENBANK-X77924; GENBANK-Y07618
ENTRY MONTH: 199710
ENTRY DATE: Entered STN: 19971105
Last Updated on STN: 20000303
Entered Medline: 19971023
ED Entered STN: 19971105
Last Updated on STN: 20000303
Entered Medline: 19971023
AB The prpR1 gene of Porphyromonas gingivalis W50 encodes the polyprotein precursor (PrpRI) of an extracellular arginine-specific protease. PrpRI is organized into four distinct domains (pro, alpha, beta, and gamma) and is processed to a heterodimeric protease (RI) which comprises the alpha and beta components in a noncovalent association. The alpha component contains the protease active site, whereas the beta component appears to have a role in adherence and hemagglutination processes. DNA sequences homologous to the coding region for the RI beta component are present at multiple loci on the P. gingivalis chromosome and may represent a family of related genes. In this report, we describe the cloning, sequence analysis, and characterization of one of these homologous loci isolated in plasmid pJM7. The 6,041-bp P. gingivalis DNA fragment in pJM7 contains a major open reading frame of 3,291 bp with coding potential for a protein with an Mr 118,700. An internal region of the deduced sequence (V304 to N768) shows 98% identity to the beta domain of PrpRI, and the recombinant product of pJM7 is immunoreactive with an antibody specific to the RI beta component. The N terminus of the deduced sequence has regional similarity to TonB-linked receptors which are frequently involved in periplasmic translocation of hemin, iron, colicins, or vitamin B12 in other bacteria. We have therefore designated this gene tla (TonB-linked adhesin). In contrast to the parent strain, an isogenic mutant of P. gingivalis W50 in which the tla was insertionally inactivated was unable to grow in medium containing low concentrations of hemin (<2.5 mg liter(-1)), and hemin-depleted cells of this mutant failed to respond to hemin in an agar diffusion plate assay. These data suggest a role for this gene product in hemin acquisition and utilization. Furthermore, the mutant produced significantly less arginine- and lysine-specific protease activities than the parent strain, indicating that there may be a regulatory relationship between tla and other members of this gene family.

L17 ANSWER 20 OF 25 MEDLINE on STN
ACCESSION NUMBER: 97047681 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8926070
TITLE: Role of Porphyromonas gingivalis protease activity in colonization of oral surfaces.
AUTHOR: Tokuda M; Duncan M; Cho M I; Kuramitsu H K
CORPORATE SOURCE: Department of Oral Biology, State University of New York at Buffalo, 14214, USA.
CONTRACT NUMBER: DE08293 (NIDCR)
SOURCE: Infection and immunity, (1996 Oct) 64 (10) 4067-73. Journal code: 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals

ENTRY MONTH: 199611
ENTRY DATE: Entered STN: 19961219
Last Updated on STN: 20000303
Entered Medline: 19961114
ED Entered STN: 19961219
Last Updated on STN: 20000303
Entered Medline: 19961114
AB Cysteine proteases, including Arg-gingipain of Porphyromonas gingivalis, have been implicated as important virulence factors in periodontal diseases. These enzymes are also involved in the hemagglutinating activity of the organisms. In order to determine the role of proteases in the colonization of the gingival margin, we have compared the attachment properties of P. gingivalis 381 with those of its Arg-gingipain-defective mutant, G-102. Interactions with gram-positive bacteria, human oral epithelial cells, extracellular matrix proteins, and type I collagen were evaluated. In all cases, mutant G-102 was deficient in attachment relative to the parental strain. The mutant's defects could be explained, in part, by the weak autoaggregation displayed by the mutant, which appeared to result from altered fimbrial expression. Both Western blot (immunoblot) and Northern (RNA) blot analyses indicated reduced expression of the major 43-kDa fimbrillin subunit in the mutant. These results suggest that Arg-gingipain may play both direct and indirect roles in the colonization of the gingival margin. In addition, fimbriae may play a direct role in interacting with some host surfaces.

L17 ANSWER 21 OF 25 MEDLINE on STN
ACCESSION NUMBER: 96255674 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8778568
TITLE: Identification of Porphyromonas gingivalis prefimbrilin possessing a long leader peptide: possible involvement of trypsin-like protease in fimbrilin maturation.
AUTHOR: Onoe T; Hoover C I; Nakayama K; Ideka T; Nakamura H; Yoshimura F
CORPORATE SOURCE: Department of Endodontics, School of Dentistry, Aichi-Gakuin University, Nagoya, Japan.
SOURCE: Microbial pathogenesis, (1995 Nov) 19 (5) 351-64. Journal code: 8606191. ISSN: 0882-4010.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199609
ENTRY DATE: Entered STN: 19960924
Last Updated on STN: 20000303
Entered Medline: 19960919
ED Entered STN: 19960924
Last Updated on STN: 20000303
Entered Medline: 19960919
AB Fimbriae of Porphyromonas gingivalis have been shown to be important as one of the virulence factors for colonization on mucosal surfaces. The gene (fimA) encoding the fimbrial subunit (fimbrilin) was overexpressed in Escherichia coli by using a bacteriophage T7 promoter-polymerase expression vector system. Analysis of the

resulting fimA gene product revealed that the prefimbrilin had a 46 amino acid leader peptide. This extremely long leader peptide was cleaved from the prefimbrilin by treatment with trypsin or P. gingivalis extracts containing trypsin-like protease activity, resulting in production of a mature fimbrilin. We also found that some transposon-induced trypsin-like protease deficient mutants of P. gingivalis exhibited deficiency in fimbriation and that one of the mutants accumulated a fimbrilin precursor possessing a 25 amino acid leader peptide in the cell. The presence of an extremely long leader peptide and the requirement for a leader peptidase with a substrate specificity similar to that of P. gingivalis trypsin-like protease for fimbrilin maturation indicate that P. gingivalis fimbrilin is a novel type that is different from fimbrilins of type I and IV families.

L17 ANSWER 22 OF 25 MEDLINE on STN
ACCESSION NUMBER: 96213021 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8631669
TITLE: Involvement of arginine-specific cysteine proteinase (Arg-gingipain) in fimbriation of Porphyromonas gingivalis.
AUTHOR: Nakayama K; Yoshimura F; Kadowaki T; Yamamoto K
CORPORATE SOURCE: Department of Microbiology, Faculty of Dentistry, Kyushu University, Fukuoka, Japan.. kojidef@mbox.nc.kyushu-u.ac.jp
SOURCE: Journal of bacteriology, (1996 May) 178 (10) 2818-24. Journal code: 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199607
ENTRY DATE: Entered STN: 19960715
Last Updated on STN: 20000303
Entered Medline: 19960702

ED Entered STN: 19960715
Last Updated on STN: 20000303
Entered Medline: 19960702

AB Arginine-specific cysteine proteinase (Arg-gingipain [RGP], a major proteinase secreted from the oral anaerobic bacterium Porphyromonas gingivalis, is encoded by two separate genes (rgpA and rgpB) on the P. gingivalis chromosome and widely implicated as an important virulence factor in the pathogenesis of periodontal disease (K. Nakayama, T. Kadowaki, K. Okamoto, and K. Yamamoto, J. Biol. Chemical 270:23619-23626, 1995). In this study, we investigated the role of RGP in the formation of P. gingivalis fimbriae which are thought to mediate adhesion of the organism to the oral surface by use of the rgp mutants. Electron microscopic observation revealed that the rgpA rgpB double (RGP-null) mutant possessed very few fimbriae on the cell surface, whereas the number of fimbriae of the rgpA or rgpB mutant was similar to that of the wild-type parent strain. The rgpB+ revertants that were isolated from the double mutant and recovered 20 to 40% of RGP activity of the wild-type parent possessed as many fimbriae as the wild-type parent, indicating that RGP significantly contributes to the fimbriation of P. gingivalis as well as to the degradation of various host

proteins, disturbance of host defense mechanisms, and hemagglutination. Immunoblot analysis of cell extracts of these mutants with antifimbrilin antiserum revealed that the rgpA rgpB double mutant produced small amounts of two immunoreactive proteins with molecular masses of 45 and 43 kDa, corresponding to those of the precursor and mature forms of fimbrilin, respectively. The result suggests that RGP may function as a processing proteinase for fimbrilin maturation. In addition, a precursor form of the 75-kDa protein, one of the major outer membrane proteins of P. gingivalis, was accumulated in the rgpA rgpB double mutant but not in the single mutants and the revertants, suggesting an extensive role for RGP in the maturation of some of the cell surface proteins.

L17 ANSWER 23 OF 25 MEDLINE on STN
ACCESSION NUMBER: 96186498 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8641806
TITLE: Selection and phenotypic characterization of nonhemagglutinating mutants of Porphyromonas gingivalis.
AUTHOR: Chandad F; Mayrand D; Grenier D; Hinode D; Mouton C
CORPORATE SOURCE: Groupe de Recherche en Ecologie Buccale, Faculte de Medecine Dentaire, Universite Laval, Quebec, Canada.
SOURCE: Infection and immunity, (1996 Mar) 64 (3) 952-8. Journal code: 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199607
ENTRY DATE: Entered STN: 19960726
Last Updated on STN: 19960726
Entered Medline: 19960716
ED Entered STN: 19960726
Last Updated on STN: 19960726
Entered Medline: 19960716
AB To further investigate the relationship between fimbriae and the hemagglutinating adhesin HA-Ag2 of Porphyromonas gingivalis, three spontaneous mutants of the type strain ATCC 33277 were selected by a hemadsorption procedure. They were characterized for hemagglutination, trypsin-like and lectin-binding activities, and hydrophobicity and for the presence of fimbriae. The presence of the 42-kDa (the fimbrilin subunit) and the 43- and 49-kDa (the HA-Ag2 components) polypeptides was investigated by immunoblotting using polyclonal and monoclonal antibodies directed to fimbriae and to the hemagglutinating adhesin HA-Ag2. Cells from two of the three mutants (M1 and M2) exhibited no or little hemagglutination activity and very low trypsin-like activity and did not show the 43- and 49-kDa polypeptides. Abnormal fimbriation in M1 was deduced from the following observations of cells grown for 18 h: absence of the 42-kDa polypeptide and of a 14-kDa polypeptide and no fimbriae visible on electron micrographs. While the cells of mutant M2, irrespective of the age of the culture, were found to lack the 43- and 49-kDa polypeptides and hemagglutination activity, the supernatants of cultures grown for 72 h had high hemagglutination and trypsin-like activities and revealed the presence of the 42-, 43-, and 49-kDa polypeptides. This suggests that M2 may be missing

some molecules which anchor the components to the cell surface. Mutant M3 showed levels of activities similar to those of the parental strain but lacked the 43-kDa polypeptide. Other pleiotropic effects observed for the mutants included loss of dark pigmentation and lower hydrophobicity. The data from this study fuel an emerging consensus whereby fimbriation, hemagglutination, and proteolytic activities, as well as other functions in P. gingivalis, are intricate.

L17 ANSWER 24 OF 25 MEDLINE on STN
ACCESSION NUMBER: 94222533 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7909537
TITLE: Construction and characterization of a fimA mutant of Porphyromonas gingivalis.
AUTHOR: Hamada N; Watanabe K; Sasakawa C; Yoshikawa M; Yoshimura F; Umemoto T
CORPORATE SOURCE: Department of Oral Microbiology, Kanagawa Dental College, Yokosuka, Japan.
SOURCE: Infection and immunity, (1994 May) 62 (5) 1696-704. Journal code: 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199406
ENTRY DATE: Entered STN: 19940613
Last Updated on STN: 20000303
Entered Medline: 19940602

ED Entered STN: 19940613
Last Updated on STN: 20000303
Entered Medline: 19940602

AB Although fimbriae of Porphyromonas gingivalis have been implicated as playing a major role in adherence to gingival tissue surfaces, no conclusive genetic evidence has yet been obtained. The fimA gene, the determinant for the major fimbrial subunit protein, was cloned and sequenced (D. P. Dickinson, M. A. Kubiniec, F. Yoshimura, and R. J. Genco, J. Bacteriol. 170:1658-1665, 1988). We undertook to inactivate the fimA gene by a homologous recombination technique and examined the fimA mutant for changes in surface properties, including production of fimbriae, adherence to human gingival fibroblasts and epithelial cells, hemagglutinating activity, and surface hydrophobicity. To inactivate the fimA gene, we disrupted a fimA clone by insertion of a DNA segment containing an erythromycin resistance (Emr) gene. This was then delivered into P. gingivalis ATCC 33277 from an Escherichia coli K-12 strain, SM10 lambda pir, by using a mobilizable suicide vector, pGP704; recombination at the fimA locus led to the isolation of a fimA mutant. Disruption of the fimA locus and disappearance of FimA production were confirmed by Southern hybridization with a fimA-specific DNA probe and Western immunoblotting with a monoclonal antibody against the FimA protein, respectively. The fimA mutant constructed failed to express long (0.5- to 1.0-micron) fimbriae from the bacterial surface and had a diminished adhesive capacity to tissue-cultured human gingival fibroblasts and epithelial cells. Observation of the bacteria adhering to human gingival fibroblasts by scanning electron microscopy revealed that the wild-type strain

had dramatic local changes in the appearance of the microvilli at the point of contact with large bacterial clumps, whereas the fimA mutant did not. In contrast, neither the hemagglutinating activity nor the surface hydrophobicity was changed in the fimA mutant. These data thus constitute the first direct genetic evidence demonstrating that the FimA protein of P. gingivalis is essential for the interaction of the organism with human gingival tissue cells through a function(s) encoded by the fimA gene.

L17 ANSWER 25 OF 25 MEDLINE on STN
ACCESSION NUMBER: 94148763 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8106316
TITLE: Inactivation of the Porphyromonas gingivalis fimA gene blocks periodontal damage in gnotobiotic rats.
AUTHOR: Malek R; Fisher J G; Caleca A; Stinson M; van Oss C J; Lee J Y; Cho M I; Genco R J; Evans R T; Dyer D W
CORPORATE SOURCE: Department of Microbiology, School of Medicine and Biomedical Sciences, State University of New York at Buffalo 14214.
CONTRACT NUMBER: DE00158 (NIDCR)
DE08240 (NIDCR)
SOURCE: Journal of bacteriology, (1994 Feb) 176 (4) 1052-9.
Journal code: 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199403
ENTRY DATE: Entered STN: 19940330
Last Updated on STN: 20000303
Entered Medline: 19940321
ED Entered STN: 19940330
Last Updated on STN: 20000303
Entered Medline: 19940321
AB Fimbrial production by Porphyromonas gingivalis was inactivated by insertion-duplication mutagenesis, using the cloned gene for the P. gingivalis major fimbrial subunit protein, fimA. by several criteria, this insertion mutation rendered P. gingivalis unable to produce fimbrilin or an intact fimbrial structure. A nonfimbriated mutant, DPG3, hemagglutinated sheep erythrocytes normally and was unimpaired in the ability to coaggregate with Streptococcus gordonii G9B. The cell surface hydrophobicity of DPG3 was also unaffected by the loss of fimbriae. However, DPG3 was significantly less able to bind to saliva-coated hydroxyapatite than wild-type P. gingivalis 381. This suggested that P. gingivalis fimbriae are important for adherence of the organism to saliva-coated oral surfaces. Further, DPG3 was significantly less able to cause periodontal bone loss in a gnotobiotic rat model of periodontal disease. These observations are consistent with other data suggesting that P. gingivalis fimbriae play an important role in the pathogenesis of human periodontal disease.

(FILE 'HCAPLUS, MEDLINE, BIOSIS, EMBASE, WPIDS, JICST-EPLUS, JAPIO, PHIC, PHIN, TOXCENTER, DISSABS, PASCAL, FEDRIP' ENTERED AT 09:07:08 ON 21 MAY 2004)
L18 73 S "FLETCHER H"?/AU AND L1 -Author

L19 30 DUP REM L18 (43 DUPLICATES REMOVED)
L20 13 S L19 AND (RECA OR REC A)

L20 ANSWER 1 OF 13 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2001:585983 HCAPLUS
DOCUMENT NUMBER: 136:197926
TITLE: The **recA** gene in **Porphyromonas gingivalis** is expressed during infection of the murine host
AUTHOR(S): Liu, Y.; **Fletcher, H. M.**
CORPORATE SOURCE: Department of Microbiology and Molecular Genetics, School of Medicine, Loma Linda University, Loma Linda, CA, 92350, USA
SOURCE: Oral Microbiology and Immunology (2001), 16(4), 218-223
CODEN: OMIMEE; ISSN: 0902-0055
PUBLISHER: Munksgaard International Publishers Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The **recA** gene in **P. gingivalis** is involved in DNA repair. To further elucidate the importance of the **recA** locus in the pathogenesis of **P. gingivalis**, the authors assessed its ability for expression in an animal host. The promoterless xa-tetA(Q)2 cassette was used in heterodiploid mutants to study **recA** promoter activity during infection. **P. gingivalis** FLL118.1 had the xa-tetA(Q)2 cassette under the control of **recA** promoter whereas **P. gingivalis** FLL119 had the cassette in the opposite orientation. Xa encodes a bifunctional xylosidase/arabinosidase enzyme (XA) and the tetA(Q)2 gene product confers tetracycline resistance. I.m. infection in a mouse model allowed the recovery of the bacteria from inguinal lymph nodes. Infusion of tetracycline in the animals permitted the enrichment **P. gingivalis** FLL118.1 over the wild-type strain, during a mixed infection. The xylosidase activity of FLL118.1 could be detected on agar plates in the presence of 5-methylumbellifiry-β-D-xyloside. No such enrichment for xylosidase activity was detected when the mixture of **P. gingivalis** W83 and **P. gingivalis** FLL119 was used to infect the mouse or cultured in vitro. These results indicated that **recA** promoter was transcriptionally active during the infection of the murine host and further support the importance of this locus during the **P. gingivalis** infection process.
REFERENCE COUNT: 28 THERE ARE 28 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L20 ANSWER 2 OF 13 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2001:399932 HCAPLUS
DOCUMENT NUMBER: 136:145959
TITLE: Environmental regulation of **recA** gene expression in **Porphyromonas gingivalis**
AUTHOR(S): Liu, Y.; **Fletcher, H. M.**
CORPORATE SOURCE: Department of Microbiology and Molecular Genetics, School of Medicine, Loma Linda

University, Loma Linda, CA, 92350, USA
SOURCE: Oral Microbiology and Immunology (2001), 16(3), 136-143
CODEN: OMIMEE; ISSN: 0902-0055
PUBLISHER: Munksgaard International Publishers Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The **recA** gene product in **Porphyromonas gingivalis** is involved in DNA repair. Further, disruption of this gene can affect the proteolytic activity and expression of other virulence factors in this organism. Since several known environmental factors can influence virulence gene expression in **P. gingivalis**, we investigated the influence of these signals on the expression of the **recA** gene in this organism. A heterodiploid strain of **P. gingivalis** (designated FLL118) containing a transcriptional fusion of the **recA** promoter region and the promoterless tetracycline-resistant gene [tetA (Q)2] and xylosidase/arabinosidase (xa) gene cassette was constructed. The **recA** promoter activity was assessed by measurement of xylosidase activity in FLL118. The expression remained relatively constant during different growth phases, at different pH levels and in the presence of DNA-damaging agents. In response to hemin limitation and in the presence of calcium there was a moderate increase in **recA** promoter activity. Temperature also affected the expression. The highest level of xylosidase activity was observed in cultures at 32°C with a decline of approx. 46% as growth temperature increased to 41°C. Reverse transcriptase polymerase chain reaction anal. revealed that this regulation may be occurring at the transcriptional level. These results suggest that expression of the **recA** gene in **P. gingivalis** W83 is responsive to several environmental signals but is not regulated by a DNA damage-inducible SOS-like regulatory system.
REFERENCE COUNT: 41 THERE ARE 41 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L20 ANSWER 3 OF 13 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2001:20261 HCAPLUS
DOCUMENT NUMBER: 134:190463
TITLE: vimA gene downstream of **recA** is involved in virulence modulation in **Porphyromonas gingivalis** W83
AUTHOR(S): Abaibou, Hafid; Chen, Zhuo; Olango, G. Jon; Liu, Yi; Edwards, Jessica; **Fletcher, Hansel M.**
CORPORATE SOURCE: Department of Microbiology and Molecular Genetics, School of Medicine, Loma Linda University, Loma Linda, CA, 92350, USA
SOURCE: Infection and Immunity (2001), 69(1), 325-335
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A 0.9-kb open reading frame encoding a unique 32-kDa protein was identified downstream of the **recA** gene of

Porphyromonas gingivalis. Reverse transcription-PCR and Northern blot anal. showed that both the **recA** gene and this open reading frame are part of the same transcriptional unit. This cloned fragment was insertionally inactivated using the ermF-ermAM antibiotic resistance cassette to create a defective mutant by allelic exchange. When plated on Brucella blood agar, the mutant strain, designated **P. gingivalis** FLL92, was non-black pigmented and showed significant reduction in beta-hemolysis compared with the parent strain, **P. gingivalis** W83. Arginine- and lysine-specific cysteine protease activities, which were mostly soluble, were approx. 90% lower than that of the parent strain. Expression of the rgpA, rgpB, and kgp protease genes was the same in **P. gingivalis** FLL92 as in the wild-type strain. In contrast to the parent strain, **P. gingivalis** FLL92 showed increased autoaggregation in addition to a significant reduction in hemagglutinating and hemolysin activities. In in vivo expts. using a mouse model, **P. gingivalis** FLL92 was dramatically less virulent than the parent strain. A mol. survey of this mutant and the parent strain using all known **P. gingivalis** insertion sequence elements as probes suggested that no intragenomic changes due to the movement of these elements have occurred in **P. gingivalis** FLL92. Taken together, these results suggest that the **recA** downstream gene, designated vimA (virulence-modulating gene), plays an important role in virulence modulation in **P. gingivalis** W83, possibly representing a novel posttranscriptional or translational regulation of virulence factors in **P. gingivalis.**

REFERENCE COUNT: 66 THERE ARE 66 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L20 ANSWER 4 OF 13 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2000:133552 HCAPLUS
DOCUMENT NUMBER: 132:165121
TITLE: Non-virulent **Porphyromonas gingivalis** mutant
INVENTOR(S): **Fletcher, Hansel M.**
PATENT ASSIGNEE(S): Loma Linda University, USA
SOURCE: PCT Int. Appl., 31 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000009156	A1	20000224	WO 1999-US18197	19990811

W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

US 6254863	B1	20010703	US 1998-133089	19980812
CA 2340070	AA	20000224	CA 1999-2340070	19990811
AU 9956724	A1	20000306	AU 1999-56724	19990811
AU 761114	B2	20030529		
EP 1105156	A1	20010613	EP 1999-943674	19990811
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
US 6585977	B1	20030701	US 2001-762618	20010209
US 2001012512	A1	20010809	US 2001-803766	20010312
US 6586227	B2	20030701		

PRIORITY APPLN. INFO.: US 1998-133089 A 19980812
WO 1999-US18197 W 19990811

AB A non-virulent, **recA** defective mutant of **Porphyromonas gingivalis** is disclosed which is deposited at ATCC under accession number 202109. Also disclosed is a method of decreasing the growth rate or reproduction rate of **Porphyromonas gingivalis** in a mammal comprising the step of administering to the mammal at least one dose of **Porphyromonas gingivalis** according to the present invention. Further, a method of preventing or treating a **Porphyromonas gingivalis** infection such as periodontitis in a mammal comprising the step of administering to the mammal at least one immunizing dose of **Porphyromonas gingivalis** according to the present invention is described. Also, a pharmaceutical composition comprising a non-virulent, **recA** defective mutant of **Porphyromonas gingivalis** is claimed.

REFERENCE COUNT: 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L20 ANSWER 5 OF 13 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2000:90228 HCAPLUS
DOCUMENT NUMBER: 132:276519
TITLE: Unaltered expression of the major protease genes in a non-virulent **recA**-defective mutant of **Porphyromonas gingivalis** W83
AUTHOR(S): Abaibou, H.; Ma, Q.; Olango, G. J.; Potempa, J.; Travis, J.; **Fletcher, H. M.**
CORPORATE SOURCE: Department of Microbiology and Molecular Genetics, Loma Linda University, Loma Linda, CA, 92350, USA
SOURCE: Oral Microbiology and Immunology (2000), 15(1), 40-47
CODEN: OMIMEE; ISSN: 0902-0055
PUBLISHER: Munksgaard International Publishers Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB **Porphyromonas gingivalis** FLL32, a **recA** mutant, was isolated during construction of a **recA** defective mutant of **P. gingivalis** W83 by allelic exchange mutagenesis. In contrast to W83 and FLL33, the typical

recA- mutant previously reported, FLL32 was non-pigmented, lacked β-hemolytic activity on blood agar and produced significantly less proteolytic activity. The proteolytic activity in FLL32 was mostly soluble Expression of the rgpA, rgpB and kgp protease genes was unaltered in FLL32 when compared to FLL33 and the wild-type strain. FLL32 exhibited reduced virulence in a murine model and partially protected the animals immunized with that strain against a subsequent lethal challenge by the wild-type strain. These results indicate that the reduced level of proteolytic activity in FLL32 may be due to a defect in the processing of the proteases. Further, immunization with a non-virulent **recA** defective mutant of **P. gingivalis** can partially protect against a lethal wild-type challenge. The results from this study suggest that the **recA** locus may be involved in expression and regulation of proteolytic activity in **P. gingivalis**.

REFERENCE COUNT: 34 THERE ARE 34 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L20 ANSWER 6 OF 13 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1997:730382 HCAPLUS
DOCUMENT NUMBER: 128:58048
TITLE: Nucleotide sequence of the **Porphyromonas gingivalis** W83 **recA** homolog and construction of a **recA**-deficient mutant
AUTHOR(S): **Fletcher, Hansel M.**; Morgan, Roderick M.; Macrina, Francis L.
CORPORATE SOURCE: Dep. Microbiology & Molecular Genetics, Loma Linda Univ., Loma Linda, CA, 92350, USA
SOURCE: Infection and Immunity (1997), 65(11), 4592-4597
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Degenerate oligonucleotide primers were used in PCR to amplify a region of the **recA** homolog from **Porphyromonas gingivalis** W83. The resulting PCR fragment was used as a probe to identify a recombinant λ DASH phage (L10) carrying the **P. gingivalis recA** homolog. The **recA** homolog was localized to a 2.1-kb BamHI fragment. The nucleotide sequence of this 2.1-kb fragment was determined, and a 1.02-kb open reading frame (341 amino acids) was detected. The predicted amino acid sequence was strikingly similar (90% identical residues) to the **RecA** protein from Bacteroides fragilis. No SOS box, characteristic of LexA-regulated promoters, was found in the 5' upstream region of the **P. gingivalis recA** homolog. In both Me methanesulfonate and UV survival expts. the **recA** homolog from **P. gingivalis** complemented the **recA** mutation of Escherichia coli HB101. The cloned **P. gingivalis recA** gene was insertionally inactivated with the ermF-ermAM antibiotic resistance cassette to create a **recA**-deficient mutant (FLL33) by allelic exchange. The **recA**-deficient mutant was significantly more sensitive to UV irradiation than the

wild-type strain, W83. W83 and FLL33 showed the same levels of virulence in in vivo expts. using a mouse model. These results suggest that the **recA** gene in **P. gingivalis** W83 plays the expected role of repairing DNA damage caused by UV irradiation However, inactivation of this gene did not alter the virulence of **P. gingivalis** in the mouse model.

REFERENCE COUNT: 39 THERE ARE 39 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L20 ANSWER 7 OF 13 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN

ACCESSION NUMBER: 2003:354507 BIOSIS
DOCUMENT NUMBER: PREV200300354507
TITLE: Non-virulent **Porphyromonas gingivalis** mutant.
AUTHOR(S): **Fletcher, Hansel M.** [Inventor, Reprint Author]
CORPORATE SOURCE: Loma Linda, CA, USA
ASSIGNEE: Loma Linda University
PATENT INFORMATION: US 6585977 July 01, 2003
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (July 1 2003) Vol. 1272, No. 1. http://www.uspto.gov/web/menu/patdata.html. e-file.
ISSN: 0098-1133 (ISSN print).
DOCUMENT TYPE: Patent
LANGUAGE: English
ENTRY DATE: Entered STN: 30 Jul 2003
Last Updated on STN: 30 Jul 2003

AB A non-virulent, **recA** defective mutant of **Porphyromonas gingivalis**. The **Porphyromonas gingivalis** strain which is deposited at ATCC under accession number 202109. Also a method of decreasing the growth rate or reproduction rate of **Porphyromonas gingivalis** in a mammal comprising the step of administering to the mammal at least one dose of **Porphyromonas gingivalis** according to the present invention. Further, a method of preventing or treating a **Porphyromonas gingivalis** infection such as periodontitis in a mammal comprising the step of administering to the mammal at least one dose of **Porphyromonas gingivalis** according to the present invention. Further, a method of preventing or treating a **Porphyromonas gingivalis** infection such as periodonitis in a mammal comprising the step of administering to the mammal at least one dose of **Porphyromonas gingivalis** according to the present invention. Also, a pharmaceutical composition comprising a non-virulent, **recA** defective mutant of **Porphyromonas gingivalis**.

L20 ANSWER 8 OF 13 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN

ACCESSION NUMBER: 2001:356355 BIOSIS
DOCUMENT NUMBER: PREV200100356355
TITLE: Non-virulent **Porphyromonas**

gingivalis mutant.
AUTHOR(S): **Fletcher, Hansel M.** [Inventor, Reprint author]
CORPORATE SOURCE: Loma Linda, CA, USA
ASSIGNEE: Loma Linda University, Loma Linda, CA, USA
PATENT INFORMATION: US 6254863 July 03, 2001
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (July 3, 2001) Vol. 1248, No. 1. e-file.
CODEN: OGUPE7. ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English
ENTRY DATE: Entered STN: 2 Aug 2001
Last Updated on STN: 19 Feb 2002

AB A non-virulent, **recA** defective mutant of **Porphyromonas gingivalis**. The **Porphyromonas gingivalis** strain which is deposited at ATCC under accession number 202109. Also a method of decreasing the growth rate or reproduction rate of **Porphyromonas gingivalis** in a mammal comprising the step of administering to the mammal at least one dose of **Porphyromonas gingivalis** according to the present invention. Further, a method of preventing or treating a **Porphyromonas gingivalis** infection such as periodontitis in a mammal comprising the step of administering to the mammal at least one dose of **Porphyromonas gingivalis** according to the present invention. Also, a pharmaceutical composition comprising a non-virulent, **recA** defective mutant of **Porphyromonas gingivalis**.

L20 ANSWER 9 OF 13 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2000:370895 BIOSIS
DOCUMENT NUMBER: PREV200000370895
TITLE: Environmental regulation of **recA** gene expression in **Porphyromonas gingivalis** W83.
AUTHOR(S): Liu, Y. [Reprint author]; **Fletcher, H. M.** [Reprint author]
CORPORATE SOURCE: Loma Linda University, Loma Linda, CA, USA
SOURCE: Abstracts of the General Meeting of the American Society for Microbiology, (2000) Vol. 100, pp. 98. print.
Meeting Info.: 100th General Meeting of the American Society for Microbiology. Los Angeles, California, USA. May 21-25, 2000. American Society for Microbiology.
ISSN: 1060-2011.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 30 Aug 2000
Last Updated on STN: 8 Jan 2002

L20 ANSWER 10 OF 13 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN

ACCESSION NUMBER: 1999:324681 BIOSIS
DOCUMENT NUMBER: PREV199900324681
TITLE: Involvement of the **recA** locus in autoaggregation in **Porphyromonas gingivalis** W83.
AUTHOR(S): Abaibou, H. [Reprint author]; Chen, Z. [Reprint author]; Liu, Y. [Reprint author]; Edwards, J. [Reprint author]; Jhuma, Z. [Reprint author]; **Fletcher, H. M.** [Reprint author]
CORPORATE SOURCE: Loma Linda University, Loma Linda, CA, USA
SOURCE: Abstracts of the General Meeting of the American Society for Microbiology, (1999) Vol. 99, pp. 49. print.
Meeting Info.: 99th General Meeting of the American Society for Microbiology. Chicago, Illinois, USA. May 30-June 3, 1999. American Society for Microbiology.
ISSN: 1060-2011.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
Conference; (Meeting Poster)
LANGUAGE: English
ENTRY DATE: Entered STN: 24 Aug 1999
Last Updated on STN: 24 Aug 1999

L20 ANSWER 11 OF 13 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1997:282157 BIOSIS
DOCUMENT NUMBER: PREV199799581360
TITLE: Virulence of **recA**-defective mutants of **Porphyromonas gingivalis** W83.
AUTHOR(S): **Fletcher, H. M.**
CORPORATE SOURCE: Loma Linda Univ., Loma Linda, CA, USA
SOURCE: Abstracts of the General Meeting of the American Society for Microbiology, (1997) Vol. 97, No. 0, pp. 101.
Meeting Info.: 97th General Meeting of the American Society for Microbiology. Miami Beach, Florida, USA. May 4-8, 1997.
ISSN: 1060-2011.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
Conference; (Meeting Poster)
LANGUAGE: English
ENTRY DATE: Entered STN: 3 Jul 1997
Last Updated on STN: 3 Jul 1997

L20 ANSWER 12 OF 13 DISSABS COPYRIGHT (C) 2004 ProQuest Information and Learning Company; All Rights Reserved on STN
ACCESSION NUMBER: 2000:43855 DISSABS Order Number: AAI9964931
TITLE: The **recA** gene in **Porphyromonas gingivalis**: Expression and regulation
AUTHOR: Liu, Yi [Ph.D.]; **Fletcher, Hansel [adviser]**
CORPORATE SOURCE: Loma Linda University (0106)
SOURCE: Dissertation Abstracts International, (2000) Vol. 61, No. 3B, p. 1195. Order No.: AAI9964931. 164 pages.
DOCUMENT TYPE: Dissertation

Searcher : Shears 571-272-2528

FILE SEGMENT: DAI
LANGUAGE: English

AB The **recA** gene product in **P. gingivalis** is involved in DNA repair. The disruption of this gene can affect the proteolytic activity and expression of other virulence factors in this organism. To further elucidate the importance of the **recA** gene in the pathogenesis of **P. gingivalis**, its in vivo and in vitro expression were investigated. In **P. gingivalis** containing the rgpA::xa-tetA(Q) 2 fusion construct [rgpA encodes for an arginine-specific protease in **P. gingivalis**, xa encodes a bifunctional xylosidase/arabinosidase enzyme and tet(A)Q2 is a tetracycline resistant gene], the expression of the xa gene could be detected both in crude extracts and on agar plates. The xa gene was used as a in this study. To investigate the influence of environmental signals, a heterodiploid strain of **P. gingivalis** containing a transcriptional fusion of the **recA** promoter region and the promoterless xa-tetA(Q) 2 cassette was constructed. The **recA** promoter activity was assessed by measurement of xylosidase activity. The expression remained relatively constant in the presence of DNA damaging agents, indicating the lack of a DNA-damage inducible SOS-like regulatory system. In response to hemin limitation and the presence of calcium there was a significant increase in **recA** promoter activity. As temperature increased, there was decreased expression of this gene, decreased proteolytic activity and a change in its distribution. The coordinate regulation of the **recA** gene with proteolytic activities may be considered an important survival strategy for this organism. In a mouse model, intramuscular infection allowed the recovery of the bacteria from inguinal lymph nodes. During a mixed infection with **P. gingivalis** W83 and FLL118.1, which contains the xa-tetA(Q)2 cassette under the control of **recA** promoter, the expression of tetracycline resistance permitted the enrichment of FLL118.1 over W83. No such enrichment was detected when a mixture of W83 and FLL119, which contains the cassette in the opposite orientation to the **recA** promoter, was used to infect the mice. These results indicated that the **recA** promoter was transcriptionally active during infection of the murine host.

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SUM **Porphyromonas gingivalis**, a black-pigmented, gram- negative anaerobe, is widely implicated as an important etiological agent of periodontal disease. This bacterium expresses several potential virulence factors (e.g., capsule, LPS, fimbriae, membrane vesicles, and hydrolytic enzymes) that may contribute to its pathogenicity. Another virulence factor, the **recA** gene, confers resistance to the oxidative stress environment of the inflammatory periodontal pocket. The **recA** gene product is a key protein in DNA repair that protects **P. gingivalis** from DNA damage induced by bactericidal reactive oxygen derivatives generated in the periodontal pocket by neutrophils and transient air exposure. Our laboratory has identified two genes, vimA and bcp, that may be part of the **recA** transcription unit and may also function in virulence. Further, the vimA-mediated virulence modulation in **P. gingivalis**, may represent a novel posttranscriptional regulation of virulence factors in this organism. Because the BCP homologue may have peroxidase function, and gingipains are involved in heme accumulation which can inactivate H2O2, it might be considered an important strategy for the organism to coordinate its oxidative stress and proteolytic activities. This importance is further supported by observation that the **recA** locus promoter is active during infection of the murine host. Moreover, the promoter activity is affected by temperature, iron and calcium which are factors known to coordinately regulate the expression of other bacterial virulence genes. Our observations, taken together, may suggest an important role for the complex **recA** locus in the survival and virulence of **P. gingivalis**. It is our hypothesis that the bcp-**recA**-vimA transcriptional unit is important for virulence and protection against oxidative stress. Our overall objective is to elucidate the molecular mechanism(s) for the vimA-mediated virulence regulation and examine the relative importance of the bcp-**recA**-vimA operon in oxidative stress resistance in **P. gingivalis**. Specific aims for the proposed research are: 1) To characterize the bcp-**recA**-vimA transcriptional unit in **P. gingivalis** W83. This will include: a) mapping the transcription initiation site; b) verifying the promoter sequence upstream of the primary start site; c) evaluating the effect of the bcp gene on the function on the **recA** and vimA genes; 2) To examine the functional significance of the vimA mutation on protease activation in **P. gingivalis** W83; and 3) To evaluate the importance of the bcp-**recA** -vimA transcriptional unit in oxidative stress protection.

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